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Report 2343

INVESTIGATION OF BEHAVIORALLY MODIFIED RATS
FOR USE IN EXPLOSIVES DETECTION SYSTEMS

by
C. Raymond V. Nolan

December 1981

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The research was designed to prove the validity of four theses: (1) Rats can detect TNT vapor via their olfactory function; (2) Trained rats will operantly signal the arrival of TNT vapor at their nares; (3) Rats may be trained en masse to function as biosensor detection systems; (4) The electroencephalogram (EEG) of trained rats contains specific signals uniquely related to their awareness of TNT vapor.

Albino male rats were equipped with four chronic indwelling brain electrodes, three of which were electroencephalograph (EEG) pick-off electrodes juxtaposed to the dura mater, while the fourth lead was a stimulus electrode embedded in the medial forebrain bundle (MFB). Electrical brain stimulation (EBS) was applied to the MFB (which has been termed a "pleasure center"), as a conditioning stimulus during training and reinforcement sessions.

Subjects were first conditioned by operant methods to associate the presence of TNT vapors with EBS and to signal awareness of the target substances by treadle pressing. When it was established that the animal clearly recognized the relationship between TNT vapor and the availability of EBS, the subjects were further conditioned, using the methods of classical conditioning, to expect EBS-gratis-on a random reinforcement schedule, only when TNT was present. Since no operant response was then required, the treadle was eliminated, and the subject was severely restrained during the training to minimize motor artifacts in the EEG. Following the initial classical protocol, randomly sequenced olfactory stimuli (TNT or neutral odorant) were delivered in simulated search paradigms. EBS was withheld during these search sessions to simulate "real world" conditions. Reinforcement sessions, periodically interspersed with searches, assured that no extinguishment of conditioning occurred.

The four theses postulated above were proven individually. It has thus been demonstrated that properly conditioned rats can, in fact, be utilized as sensory elements in biosensor explosives detection systems.

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METRIC CONVERSION FACTORS

Approximate Conversions to Metric Measures

Symbol	When You Know	Multiply by	To Find	Symbol
LENGTH				
in.	inches	2.5	centimeters	cm
ft	feet	30	centimeters	cm
yd	yards	0.9	meters	m
mi	miles	1.6	kilometers	km
AREA				
in. ²	square inches	6.5	square centimeters	cm ²
ft ²	square feet	0.09	square meters	m ²
yd ²	square yards	0.8	square meters	m ²
mi ²	square miles	2.6	square kilometers	km ²
	acres	0.4	hectares	ha
MASS (weight)				
oz	ounces	28	grams	g
lb	pounds	0.45	kilograms	kg
	short tons (2000 lb)	0.9	metric ton	t
VOLUME				
tsp	teaspoons	5	milliliters	ml
tbsp	tablespoons	15	milliliters	ml
in. ³	cubic inches	16	milliliters	ml
fl oz	fluid ounces	30	milliliters	ml
c	cups	0.24	liters	l
pt	pints	0.47	liters	l
qt	quarts	0.95	liters	l
gal	gallons	3.8	liters	l
ft ³	cubic feet	0.03	cubic meters	m ³
yd ³	cubic yards	0.76	cubic meters	m ³
TEMPERATURE (exact)				
°F	degrees Fahrenheit	5.9 (after subtracting 32)	degrees Celsius	°C

Approximate Conversions
from Metric Measures

Symbol	When You Know	Multiply by	To Find	Symbol
LENGTH				
mm	millimeters	0.04	inches	in.
cm	centimeters	0.4	inches	in.
m	meters	3.3	feet	ft.
m	meters	1.1	yards	yd
km	kilometers	0.6	miles	mi
AREA				
cm ²	square centimeters	0.16	square inches	in. ²
m ²	square meters	1.2	square yards	yd ²
km ²	square kilometers	0.4	square miles	mi ²
ha	hectares (10,000 m ²)	2.5	acres	
MASS (weight)				
g	grams	0.035	ounces	oz
kg	kilograms	2.2	pounds	lb
t	metric ton (1000 kg)	1.1	short tons	
VOLUME				
ml	milliliters	0.03	fluid ounces	fl oz
ml	milliliters	0.06	cubic inches	in. ³
l	liters	2.1	pints	pt
l	liters	1.06	quarts	qt
l	liters	0.26	gallons	gal
m ³	cubic meters	35	cubic feet	ft ³
m ³	cubic meters	1.3	cubic yards	yd ³
TEMPERATURE (exact)				
°C	degrees Celsius	— 9/5 (then add 32)	degrees Fahrenheit	°F

INVESTIGATION OF BEHAVIORALLY MODIFIED RATS FOR USE IN EXPLOSIVES DETECTION SYSTEMS

1. INTRODUCTION

1. Synopsis of the Experiment. The research discussed in this report is concerned with improving the operational reliability of animals employed as the sensory element in explosives detection systems. Four basic theses were investigated, and the research results in each of these areas represented an original contribution to the developing field of biosensor detection systems. These theses are:

Thesis A: It is postulated that rats can detect, via their olfactory sensory function, some component of the military explosive 2,4,6-trinitrotoluene (TNT) in minute quantities.

Thesis B: It is postulated that if Thesis A is true, then proper behavioral conditioning can cause the animals to operantly signal their awareness of the presence of some effluent of TNT in the ambient air.

Thesis C: It is postulated that if Thesis B is true, then it should be possible to train several subjects simultaneously (as opposed to the traditional method of individual sequential conditioning of multiple test subjects) using a semi-automatic test station employing operant and classical conditioning paradigms.

Thesis D: It is postulated that if Thesis B is true, then it may be possible to elucidate some statistically significant change in the cortical electroencephalogram (EEG) of the test subjects after the act of detection has occurred and prior to operantly signaling—or, in the absence of the opportunity to operantly signal—the detection. Phrased differently, it should be possible to classically condition the test subjects to anticipate the reward that was heretofore forthcoming only as a consequence of an operant. The state of cerebral arousal resulting from the anticipation should then result in an anticipatory evoked spectral change (AESC) in the cortical EEG.

The overall research effort spanned a period of over 4 yr during which two basically similar experiments—hereafter simply labeled Experiment I and Experiment II—were performed. The research demonstrated that each of the four postulates is true insofar as laboratory experiments can verify these postulates. For the most pragmatic verification, it will be necessary to train a large number of subjects; equip a portable detection system with the subjects, suitable microprocessor devices, air inlet devices,

alarm devices, etc.) and exercise a series of double blind field tests similar to those employed with other subjects.¹ Only after successful completion of such tests can one state unequivocally that a new sensor system has been developed.

2. Research in Sensory Systems. In this decade, mankind faces a critical threat brought about by the shift in the effectiveness of the activities of the criminal element of society relative to that of the law enforcement and regulatory agencies of all nations. Crimes against the individual and against society in general are escalating for reasons which appear to be beyond simple rationalization. The causes are not germane here, but the appearance of increasingly sophisticated activities on the part of the contrasocial elements of society demands the conception, development, and deployment of truly effective methods for diminishing the effectiveness of criminal operations. This research is the direct result of one step toward this goal.

One of the more chilling criminal threats today is the potential for so-called radical or terrorist groups to deploy and detonate explosive devices in public areas with virtually zero probability of predetonation detection of the device. The ultimate horror—the use of nuclear explosives by these groups—may occur within the next decade unless some quantum leaps forward are soon available in the science/art of explosives detection. The probability of “nuclear terrorism” depends upon the effectiveness of the safeguards which shield fissionable materials from ready access to skilled intruders who would almost certainly find it necessary to use high explosives to gain access to these materials. It is evident that *truly effective* explosives detectors are desperately needed for use in those public areas susceptible to threat and in the access pathways to the strong, but not impenetrable, nuclear storage areas.

In spite of the importance to society of adding to the armamentarium of law enforcement elements, there are only a few hundred persons known to be actively engaged in research and development activities directly related to explosives sensing systems. This small scientific community, faced with a task which has hitherto proved to be virtually impossible (on a practical basis), seems to be slowly devolving into two group philosophies of detection research. The dichotomy thus generated resolves to the following research categories:

Group A: Physicochemical sensors.

Group B: Biosensors.

Each group is apparently strongly persuaded that their approach is best suited to the purpose, and, while there is normally little active opposition each to the other, there is also little mutual interest.

¹ Nolan, R. A., and Grayson, D. L. “Mine Detecting Canines.” Report 2207, U.S. Army, MERRIAM, Ft. Belvoir, Arizona (1977).

3. **Physico-Chemical Sensor.** Obviously, the research described here falls into Group B, and I have attempted to maintain a high degree of objectivity in the rationale for my selection of this group as the one most likely to achieve the detection goal in the near future. Prior to discussing the merits of Group B sensor systems, it would be well to consider briefly those techniques which lie in Group A. Group A embraces some conventional techniques which are nothing more than attempts to adapt some item of conventional laboratory equipment to field use. Virtually hundreds of schemes have been put forth which purport to provide some improvement in the efficacy of the gas chromatograph (GC), the mass spectrograph (MS), the plasma chromatograph (PC), or the combinations of these systems (GS/MS and PC/MS). These are instruments which function well in the laboratory but which often prove to be impractical for use in field service as explosives detectors.

The reasons for this low utility are pragmatic. This class of instrumentation is either very fragile and, hence, not truly transportable (as an explosives detector generally MUST be), or if they are made to be rugged and thus are transportable, the cost is unacceptable for general use even when economies of scale are considered. But most significantly, these instruments (except for the PC), whether rugged or fragile, cannot by their nature be made to operate in real time. This is a fatal flaw, since after-the-fact explosives detection, in many cases, is fatal to the search device and to any human operator unfortunate enough to accompany it.

In addition to the systems described above, Group A also contains a vast array of schemes which use some form of electromagnetic detection. Numerous attempts at explosives detection have used techniques ranging from sophisticated adaptations of CW radars, short- and long-pulse radars, x-rays, and Gamma-ray and neutron radiation to simple schemes, such as the balanced RF (radio-frequency) bridges seen in contemporary commercial and early military devices. These devices will usually detect in real time (< 1 s), but there are other aspects of performance which render them generally useless. These problems are well expressed in the often-heard phrase, "detection is not really the problem—the problem is one of identification." Indeed, most of these systems can, with various degrees of success, detect anomalies in the ambient environment, but they will not uniquely detect *explosives*, and thus they are essentially useless in the desired detection service where high detection probability must be coupled with a low false alarm probability if searches are to be completed in a timely manner. This latter consideration leads to the two final factors which must be considered relative to the techniques of Group A. These are the factors of sensitivity and specificity.

There are numerous relatively innocuous chemical compounds which have molecular structural components similar to those in explosives, and a practical detection device must be capable of selecting some physical factor or group of factors which will

unequivocally indicate that the suspect substance is or is not one of the inimical set. Further, the device must be able to function with a reasonable false alarm rate (< 15 percent) in an environment where the effective signal-to-clutter ratio may be much less than unity, and thus high specificity is absolutely an essential operating parameter if low false alarm probability is to be obtained. Finally, the system must be capable of sufficient sensitivity to permit detection while the target is not immediately proximal to the detector. In practice, the range of detection should be measured in feet, not in inches, and this stringent requirement, coupled with the need for real time detection annunciation, presently eliminates most physico-chemical schemes from any serious consideration for use as portable explosives detection systems, or for detectors for service in which the target object is in motion (e.g., humans in portals, corridors, etc.).

4. Biosensor Research. Biosensors, the system of Group B, show promise of the ability to overcome many of the objectionable characteristics inherent in the Group A systems, but they are not without serious flaws.

In order to elucidate the arguments for biosensors, one must first define the term biosensor as it applies to the research. For the purposes of this research, a biosensor is defined as a sensor element (or a total sensory system) which either consists of a living organism or which is an in vitro application of some life process. The latter category embraces systems employing enzymatic chemistry, immuno-enzymatic techniques, olfactory receptor protein utilization schemes, and a few other experimental approaches which will not be discussed here.

The former (in vivo) category directly addresses the use of living creatures which are in some manner structurally or behaviorally modified (or both) to serve either as a sensory element or as a total detection system.

The most popular application of animals to date has been the use of behaviorally modified dogs which have demonstrated astonishing performance in thousands of documented tests of detection employing a variety of targets such as drugs, explosives, humans, and so on. While dogs have performed extremely well, even the most dedicated biosensor enthusiast must admit that the phrase "extremely well" is an eponym which may be undeserved, since presently their overall performance leaves much to be desired.

The basic objection to dogs and other intact animals which are behaviorally conditioned to detect some substance is that their performance varies from hour to hour and from day to day, and the user is never certain that the ultimate performance is being extracted from the animal in any given search. There is no reason to believe that these animals are always willing or even able to perform at maximum sensitivity and specificity. In fact, Army canine research programs have demonstrated² that quite often a hitherto

² Nolan, R. A., and Gravette, D. L., "Mine-Detecting Canines," Report 2217, U. S. Army, MERRIAM, Ft. Belvoir, Virginia (1977).

dependable animal will suddenly choose not to function as a detector, and yet there is little or nothing apparent in the observed behavior to indicate that this biosensor is in a "failure mode." Such failures have been disastrous to the point that it might have been better to have used a cautious human search or some slow but possibly more dependable biosensor system such as an enzymatic detector in preference to the living animal.

In view of these negative aspects of animal behavior, the unlimited use of such systems has been viewed as suspect by many potential users. Additionally, animals may exhibit totally unexpected traits in tense situations, such as on battlefields or in crowded areas or where the geophysical environment is unusual.

If there were methods available to eliminate the effects of indifference, fear, and whatever other emotion might cause failures in performance, then animals might well be the detector system of choice in many scenarios. Certainly there can be no valid argument against the statement that, for explosives detection service in such diverse environments as in buildings, on urban streets, on bridges, along railroad tracks, in vehicles, in aircraft, in tunnels, and so on, the animal with its proven ability to ignore most clutter, is the best all-purpose explosives detection system currently available.³

The search for a method of eliminating most, if not all, of the undesirable aspects of intact-animal sensors culminated in the research program which is the subject of Thesis D.

First, it was necessary to select a suitable animal (see paragraph 5, "Test Subjects") which, by its nature, would not manifest many of the undesirable character traits noted above. Then it was necessary to devise a scheme whereby this animal could be induced to sense target substances (detect explosive effluents) with unprecedented singleness of purpose, while at the same time maximally exercising its natural ability to discriminate the desired target substance in the presence of extremely heavy clutter.

A basic assumption at the initiation of the research was that the ultimate overall system performance would be attained if it were possible to elucidate the presence of a unique event in the cortical neural activity (anticipatory evoked spectral change, or AESC) of a suitably conditioned subject, following the introduction of explosive effluents into the air proximal to the subject. This concept assumed that the anticipatory event would occur as the subject contemplated carrying out an operant act of annunciation which, during its training, had evoked a highly desirable reward when the target

³ To even approach equal detection probabilities in a single "black box" would require a complex array of many chemical systems of exquisite and as yet unattainable sophistication. This concatenation, in addition to being too expensive even to contemplate for general service, would very likely be too large and heavy to be considered portable. Even the use of biosensors such as the enzyme systems noted earlier are not necessarily utile for day-to-day use in such service.

substance was present. If this event could be unequivocally identified, then an act of annunciation (an operant) on the part of the animal would become unnecessary, and the AESC, alone, properly identified by a microprocessor, would signal the presence of a target substance with high reliability.⁴

Obviously, with the elucidation of the AESC, there would exist a firm basis for the development of an explosives detection system which used an intact animal (thus requiring no elaborate support system) as the sensory element.

It should not be inferred from the foregoing that the unavailability of some form of AESC would render suitably trained animals useless. It is quite feasible to use two or more small animals in a portable device wherein each animal would operantly signal that it has detected a target substance. The probability that three animals would signal a false positive within the period of 15 s or so is small—possibly 0.10 or less. Therefore, such a system using majority logic decision criteria would be nearly as effective as a system using one animal which delivered an AESC to a microcomputer. However, the logistical and other practical considerations inevitably lead to the choice of a single-animal system if at all possible.

5. Test Subjects. In the broadest sense, the term "biosensor" relates not only to the animal and vegetable Phyla but also to the various in vitro utilizations of life processes. It was necessary early in the conceptual phase of this program to narrow the field of consideration to embrace only those biosensors which could show a potential for near-future utility in a detection research program. For this reason, only living sensors were evaluated, and this category was quickly reduced to include only the animal Phyla.

Within this grouping, the optimum choice of subject was not clearly evident on first consideration, for there are reports in the literature which ascribe all manner of sensory capabilities to nearly every class of animal.

Since the research program was not designed to be an investigation of every possible parameter of every conceivable biosensor system, it was necessary to establish certain criteria prior to the selection of a test subject, and the first, and possibly the most

⁴ It is perhaps pertinent to note at this point that virtually any substance can be the target substance. The use of TNT in this research was predicated by the specific needs of the US Army, which sponsored the overall program, but Army research with dogs has shown that the use of other targets, such as various narcotics, handguns, and so on, will result in probabilities of detection equal to that observed with explosives if the test subjects are suitably trained to the desired target.

important, criterion had to do with the modality of detection (see paragraph 6, "A Question of Modality"). An extensive effort on a prior program⁵ suggested that olfaction was the most suitable modality to consider for this research, and consequently only those animals which could be shown to exhibit macrosmatic characteristics were considered as candidates for experimental subjects.⁶

This requirement narrowed the field of consideration rather quickly. Reptiles were immediately excluded since they appear to exhibit high sensitivity for electromagnetic radiation in the infrared region, but there was no evidence of osmatic excellence.

Most birds have been shown to exhibit no macrosmatic tendencies⁷ which is not surprising since they, as man, have evolved such that vision is the dominant sense. Insects, the Bombyx moth in particular, have been shown to exhibit an astonishing olfactory sensitivity on the order of 10^{10} mol fraction,^{8,9} but this great sensitivity apparently is limited to the detection of the Bombyx female sex pheromone. Indeed, the elegant experiments with these moths at Max Plank Institut failed to demonstrate this high sensitivity for any substance other than one specific pheromone (although there was some evidence of unusual sensitivity for certain nutrients). Since there was no evidence available to indicate that any insect would display any interest in or extreme sensitivity for any explosive substance, the invertebrate animals were excluded from serious consideration.

The second important criterion related to the ease with which the candidates could be trained, or conditioned, to reliably perform a specific task. Obviously, those animals which previously had been shown to be easily trained and which had demonstrated high reliability in performing to the training protocol were given primary consideration.

In the combined factors of these two criteria led to the selection of mammals as the optimum class of subjects. Then, it was necessary to select, from this enormous class, the best subject for this particular research program. Nearly all mammals appear to exhibit macrosmatic capability, except the order of primates. (Even in this order, only *Homo sapiens* is not capable of fairly high osmatic sensitivity.) Further, most mammals,

⁵ Nolan, R. A., and Grayson, D. L., "Mine-Detecting Canines," Report 2217, U. S. Army, MERRIAM, HI, Belton, Virginia (1977).

⁶ There is fragmentary evidence that some sensory modality other than olfaction may exist in explosives and narcotics detecting dogs, but at present these data are too vague to be meaningful.

⁷ Wenzel, R. M., "Olfaction in Birds," In Biedler, L. M., ed., *Handbook of Senses and Physiology*, Series 1, Vol. 12, New York (1971).

⁸ Schneider, D., "Insect Olfaction: Deciphering System for Chemical Messages," Science 166:1031-32 (1969).

⁹ Schneider, D., Block, R. C., Block, E., and Prasner, E., "Die Reaktion der weiblichen Bombyx mori auf einen homölog und einen isomeren elektrophoretischen und verhalten," Zeitschrift für Physiologie 114:1-10 (1967).

¹⁰ Schneider, D., Kasang, G., and Karsch, K. E., "Bestimmung der molekularen Struktur eines Bombyx mori - sexuellen markierten homölogol," Naturwissenschaften 53:61-62 (1966).

even those of the order Rodentia, can be easily trained to perform reliably a great variety of tasks; and it must be noted that these creatures appear to exercise some remarkably advanced reasoning in achieving their performance.

Until very recently, there have been no attempts to quantify the limit of sensitivity of any mammal to a specific olfactory stimulus. The reasons for this dearth of research appear to be lack of interest (at the financial level, not the scientific level) and lack of adequate laboratory apparatus with which to measure this quantity. The fate of the current sensitivity research is not a suitable subject for speculation here, but the problems of technique and instrumentation are formidable, and some time may pass before incontestable data are available which show the true olfactory sensitivity of any animal to substances manifesting low vapor pressure (such as TNT, heroin, etc.).

In the interim, the mammal must be judged by the results of careful testing against a small set of target substances. These results when compared to those obtained by any other detection scheme in the area of explosives and narcotics detection (admittedly a somewhat subjective process at best) show much to recommend mammals as biosensors. Specifically, most of these animals appear to exhibit a very high sensitivity to a wide spectrum of target substances while maintaining a relatively low false alarm rate. Since the terms "very high" and "relatively low" are subjective, it is pertinent to state that in *one series of tests* (in one research program¹¹) dogs exhibited 0.90 probability of detection and 0.15 probability of false alarm—an excellent performance record for any detection system, especially in view of the fact that the odorant substances were contained in Army land mines which are waterproof (and ostensibly airtight) devices. One must also consider the fact that these mines were buried to a depth of at least 3 in. (top surface of the mine to the average surrounding land surface).

An enormous body of non-specific (or anecdotal) data relative to mammalian olfactory detection is in general agreement with these results, and these anecdotes must be given due credence as a selection criterion, especially since so little truly objective data are presently available.

Having thus chosen the class of mammals for detection service, it remained to choose the specific animal best suited to the research program. A great number of genera and species of animals have been used in experiments related to explosives detection, as Table 1 may indicate.

¹¹ Nolan, R. V., and Gravette, D. L., "Mine-Detecting Canines," Report 2247, U. S. Army, MERADCOM, Ft. Belvoir, Virginia (1977).

Table 1. Animals Used in Explosives Detection Studies*

Animal	Number Used	Genus and Species
Badger	1	<i>Taxidea taxus</i>
Coatimundi	2	<i>Nasua nasua</i>
Coyote	4	<i>Canis latrans</i>
Coyote/beagle cross	2	<i>Canis latrans</i> X <i>Canis familiaris</i>
Deer (white tail)	2	<i>Odocoileus virginianus</i>
Domestic Dog	83	<i>Canis familiaris</i>
Ferret	4	<i>Mustela putorius</i>
Fox (Red)	4	<i>Vulpes vulpes</i>
Hog (Red Duroc)	4	<i>Sus scrofa domestica</i>
Javelina	3	<i>Tajacu pecari</i>
Miniature Pig	4	
Opossum	3	<i>Didelphis virginiana</i>
Raccoon	4	<i>Procyon lotor</i>
Skunk (spotted)	1	<i>Spilogale putorius</i>
Skunk (striped)	2	<i>Mephitis mephitis</i>
Skunk (hog nosed)	1	<i>Conepatus mesoleucus</i>
Timber Wolf	4	<i>Canis lupis</i>

* Extracted from US Army MERAIXOM Report 2217, "Mine-Detecting Canines," dated 1977.

In addition to the foregoing set, the US Navy has experimented extensively with Cetacea (the bottlenose porpoise) and other marine animals, and several other agencies of the US (and some foreign governments) are currently investigating still other mammals for explosives and narcotics detection service.

As noted earlier, the dog has been, and continues to be, the animal most often selected for detection service. The criteria for this choice may be largely based upon emotion rather than reason, since dogs have numerous highly undesirable traits and very little of a practical nature can be done to increase their reliability.¹²

To be useful in most searches, the detector should be man-portable and disguisable as some ordinary item, and this operational parameter suggests the use of a small (<500 g) mammal as the sensory element. This animal must be macrosmatic and of a genera which does not evoke social comment should invasive processes, such as brain electrostimulus be used in controlling it; and this caveat leads to the inevitable conclusion that some small "laboratory" animal would be ideal for this type of service if it could be induced to function as a sensory system.

¹² Undoubtedly, their performance could be vastly improved by the use of electrical brain stimulus, but this procedure, or any form of physically invasive behavioral control in the case of "man's best friend," is probably unacceptable to contemporary society.

It is doubtful if any animal relies more on olfaction than the rat, which probably has survived as a mammalian order largely because of its macrosomatic capability. The rat seeks water, food, mate, and domicile (in that order) by smell, and there is evidence that a blinded and deafened rat can survive for long periods in its natural environment—a feat probably unequalled outside the order Rodentia.

An essential criterion to be met by the test subject is unit-to-unit reproducibility. Most species of rats used in research are ideal since a tremendous degree of in-breeding has resulted in strains which are remarkably alike both structurally and emotionally.¹³

Following this logic, the rat (and within the species, the Sprague-Dawley strain) ultimately was chosen for this work. These animals, when adult, achieve a maximum weight of about 500 g, attain a body length of about 20 cm, have a relatively docile nature, and evidence no intense natural fear of man. As in the case with most Rodentia, these animals exhibit no affection toward or dependence upon humans, and there is generally a reciprocal response on the part of *Homo sapiens*. This final factor is of significance since some mammals (most notably dogs) obviously need emotional as well as sustentative support from man in order to function as a detection system, and this dependence can cause serious problems in deployment. For example, if the regular handler is not available, many dogs either refuse to search or will perform so poorly that any alert signal is suspect, and the detection probability is indeterminate.

Further, the human tendency toward emotional ties with animals is often inimical to detection service as witnessed by the repeated failure of dog handlers to follow prescribed reward regimens in Army tests. This practice perhaps satisfied some need on the part of the handler, but it quickly led to an increased false alarm probability during long test sequences, since the dogs soon realized that a reward was to be offered at each alert signal even if there was no target substance present. There is no tendency toward this improper reward syndrome in the case of rat handlers, and rats do not appear to distinguish between individual humans, so that there is no evident variance in performance with any number of different test operators.

6. A Question of Modality. The use of animal biosensors immediately evokes inquiry as to the nature of the sensory process involved in detection of the target substance. It is not a simple matter to define, and much controversy exists as to the exact nature of the process or processes at work in those animals—principally dogs—which have accumulated a lengthy performance history.

A high degree of reproducibility was observed repeatedly during the test, as witnessed by the success rate of electrode implant and by the behavioral results. Needless to say, the unit-to-unit structural similarity allows the available stereotaxic atlas of the brain for rats to be more detailed than those of other animals.

It is difficult for some observers to imagine that a sensory modality other than olfaction is in operation during tests where there are no visual clues discernable to humans and where the target emits no sound evident to humans, but the existence of an unrecognized sensory modality in certain animals must be accepted as possible, if not likely (twitness the recent discovery of electrical gradient sensors in some fish). At present, however, there is absolutely no current *scientific* basis for such a premise in the case of mammals. One might speculate that mammals may have a magnetic sensory modality similar to that demonstrated in pigeons, but none of the test data available to date indicate that any magnetic anomalies are caused by the presence of a small amount of pure explosive of any type. Nor is there any detectable electrostatic influence exhibited by these substances. In short, no electromagnetic disturbance can be attributed to these particular classes of test target substances by state-of-the-art instrumentation, but then, in most cases, state-of-the-art physico-chemical instruments cannot detect targets which animals can readily locate.

The arguments favoring olfaction as the single detection modality are really not much more persuasive than those which elucidate the likelihood of some mysterious modality to which humans are not privy. However, the balance of current opinion—and it can only be opinion—seems to favor olfaction as the dominant, if not the only, sensory modality used by explosives detecting animal biosensors, and the research was structured accordingly. The performance of the test rats appears to attest to the validity of this premise, since the test subjects readily detected the presence of TNT vapor in the ambient (test cage) air, and yet they were at all times physically removed from the test odorant substances by at least 1.5 m with the only conceivable communication between the subjects and the target substance being a contained air stream directed from the odorant substance. This fact argues strongly for the single detection modality of olfaction, at least in the case of rats.

7. Olfaction. A physiologist addressing a group of medical students was once heard to define olfaction as "the vacuum of physiology." This is an apt definition as is evident after a few hours of cursory examination of the reference material cited in the bibliography. The literature of olfaction exhibits a striking contrast to a similar sampling of the works related to visual physiology. The texts dealing with olfaction are highly speculative in nature and tend to offer little concrete evidence as to the nature of the neural processing of this chemical sense, whereas any current text in general physiology will define the visual system in great detail morphologically, and may even delve somewhat into the subjective nature of the visual experience.

Homo sapiens evidently made a collective evolutionary "decision" to adopt vision as its dominant sensory modality, and thus vision became critical to species survival, while hearing, at least in modern man, is much less significant to survival; and olfaction and gustation are hierarchically far below audition in survival significance.

The order of survival significance in man is also the apparent order of research significance, and thus the relatively minor knowledge of the physiology of olfaction is understandable, but none the less disturbing for those scientists involved in biosensor detection system research.

There is little merit in incorporating a profound discussion of the current knowledge of olfaction in this report, but a few observations may be pertinent in view of the fact that the research team collectively presumed olfaction to be the modality of detection used by animal biosensors.

There is much controversy concerning the absolute sensitivity of animals to odorant stimuli. One philosophy holds that animals—at least macroscopic animals—possess an inherently greater receptor sensitivity than that of man, while another persuasion suggests that the basic sensitivities of all mammals are similar, but that a vast difference exists in post-detection signal processing.

Those who embrace the concept of inherently greater receptor sensitivity point out that the olfactory mucosa in macroscopic animals is far greater in area (when corrected for body size) than that found in humans. They note the presence of the vomeronasal epithelium which adds to the total sensory area in animals (this organ is vestigial in man), and finally, this group notes that the ratio of the mass of the olfactory bulb and the mass of the related neural structures in the cortex and limbic system to the total non-olfactory cortical mass is far greater in lower animals than in man. The principal contention of this group is that the greater mucosal area equates with a greater probability of capture of odorant molecules at a receptor site, and that this fact alone could account for the apparent divergence in sensitivity. Some proponents of this concept argue that since olfaction is vital to survival in many mammals, the process is further augmented in these species by receptor sites of superior sensitivity at the molecular level of environmental interface.

Certainly one cannot argue the points of morphology. The ratio of olfactory neural structure mass to total cortical mass is indeed greater in macroscopic animals than in man, and the receptor site area is certainly relatively larger. However, there is currently no conclusive evidence of superior sensitivity at the level of the receptor proteins of infra-human mammals. In fact, there is no universal agreement as to the nature and function of the actual receptor sites, and thus, arguments as to the relative sensitivity of receptor sites are somewhat specious at best.

Just as there is no accepted model for an olfactory receptor, there is also no agreement as to the nature of an odorant. Over the past century, several prominent physiologists have attempted¹⁴⁻²⁹ without success to classify odors. Some scientists have tortuously argued that as few as four basic odors could account for all known olfactory sensations in man since there are supposedly only four basic gustatory stimuli, and yet man can identify thousands of individual taste sensations. Other opinions have argued that there are unlimited stimuli, each of a different nature, which somehow react with a set of (possibly) identical receptor proteins in such a manner as to produce the variety of observed sensations.

The second philosophy noted above has little relative concern for the exact process of stimulation, and holds simply that the receptor mechanism is most likely the same in all mammals, however it functions, and hence all should exhibit the same sensitivity expressed as a mol fraction *at the receptor site*. Thus, man and other animals may be equal in ability at this point in the olfactory system. If this is true, then the basic difference in the ability of man and macrosmatic animals to detect and define an odorant must lie entirely in the nature of the post-detection signal processing. Cain³⁰ has elaborated an appealing simile wherein he likens the ability of macrosmatic creatures to detect and identify smells to man's ability to visualize in a cube and then to rotate this cube so that a mental inspection of all possible aspects of the cube relative to its imagined environment is made. An animal may well have the ability to examine an olfactory stimulus

- 14 Adrian, E. D., "The Sense of Smell," *Advances in Science* 1:287-92 (1948).
- 15 Adrian, E. D., "Sensory Messages, and Sensation: The Response of the Olfactory Organ to Different Smells," *Acta Physiologica Scandinavica* 29:5-15 (1953).
- 16 Amoore, J. E., "Stereochemical Theory of Olfaction," *Nature* 198:271-72 (1963).
- 17 Amoore, J. E., "A Plan to Identify Most of the Primary Odors," *Proceedings of the Third International Symposium on Olfaction and Taste*, Rockefeller University Press, New York (1969).
- 18 Ashton, E. H., "Olfactory Acuity in the Dog," *Nature* 179:1069-70 (1957).
- 19 Cain, W. S., "Differential Sensitivity for Smell: Noise at the Nose," *Science* 195:796-98 (1977).
- 20 Cain, W. S., "Olfaction and the Common Chemical Sense: Some Psychophysical Contrasts," *Sensory Processes* 1:57-67 (1976).
- 21 Gesteland, R. C., "Chemical Mechanisms and Neural Coding in Vertebrate Chemosenses," *Aerospace Medical Research Laboratory Report No. AMRL-TR-70-117*, Wright-Patterson Air Force Base, Ohio (1971).
- 22 Gesteland, R., Lettvin, J. Y., Pitts, W. H., and Chung, S. H., "A Code in the Nose," In: Oestreicher, H. L. and Moore, D. R. (eds.), *Bionics Symposium*, Bouldon and Breach, New York (1968).
- 23 Hainer, A. G., "An Information Theory of Olfaction," *Annals of the New York Academy of Science* 116:177-81 (1964).
- 24 Johnston, J. W., "An Application of the Steric Odor Theory," *Georgetown Medical Bulletin* 17:40-42 (1963).
- 25 Moskowitz, H. R., "Standardized Procedure for Expressing Odor Intensity," *Chemical Senses and Flavor* 1:235-7 (1974).
- 26 Robertson, P. W., "Do Smells Have Frequencies?" *New Scientist* 11:419 (1961).
- 27 Robinson, D. Z., "Theory of Smell," *New Scientist* 5:714 (1959).
- 28 Rosenzweig, M. R., and Bennett, E. L. (eds.), "Neural Mechanisms of Learning and Memory," The MIT Press, Cambridge (1976).
- 29 Zwaardemaker, H., "Die Physiologie des Geruchs," Wilhelm Engelmann, Leipzig, Germany (1895).
- 30 Cain, W. S., "Olfaction and the Common Chemical Sense: Some Psychophysical Contrasts," *Sensory Processes* 1:57-67 (1976).

in this abstract manner and by this means to exclude all, or nearly all, extraneous inputs from the ambient environment. Dr. Cain postulated that such abstraction may be the proper function of the large olfactory neural system found in macrosmatic animals. This concept allows a rational explanation of the observed functional ability of animals to detect one specific substance in the midst of vastly more prevalent odorants.³¹ Possibly, then, even the humble rat possesses an ability for abstract thinking in olfactory terms which humans cannot readily imagine.

8. Conditioned Behavior. The phrase "conditioned behavior" is frequently used by scientists to define a learning process through which an animal—be it worm or man—can be caused to develop a specific pattern of behavior in response to a specific set of stimuli.

In the face of considerable dispute, Rene Descartes, in 1664, espoused the then revolutionary concept that every action of every organism is the necessary result of the application of some external stimulus. Two centuries later in 1898, E. L. Thorndike³² began the first recorded scientific investigations of the response of various animals to environmental stimuli. Shortly after this, in 1907, I. P. Pavlov^{33,35} also began to investigate the reaction of animals to controlled stimuli. In the 1920s and 1930s, Thorndike's work was expanded by S. Miller and J. Kanorski in France and by B. F. Skinner^{36,39} in the United States.

Thorndike originated a protocol which is termed "instrumental conditioning" by some authorities and "operant conditioning" by others. The work of Skinner in this area is basically an elaboration of the pioneer efforts of Thorndike, and the term operant conditioning was apparently introduced by Skinner in 1938.³⁴ Since this term is perhaps more popular in current terminology, it will be used throughout this report with the explicit understanding that it is totally interchangeable with the term instrumental conditioning.

³¹ Witness the many anecdotes relating to the pursuit of errant humans by tracker dogs which have resulted in flawless tracking of one individual through an incredible field of strong odorants.

³² Thorndike, E. L., "Animal Intelligence: An Experimental Study of the Associative Processes in Animals," Psychological Monographs 101 (1904).

³³ Pavlov, I. P., "Conditioned Reflexes: An Investigation of the Physiological Activity of the Cerebral Cortex," Translated and edited by Ansel G. V. Dover Publications, New York (1960).

³⁴ Pavlov, I. P., "Lectures on Psychology and Psychiatry," The Citadel Press, New York (1962).

³⁵ Pavlov, I. P., "Lectures on Conditioned Reflexes," Translated and edited by Gantt, W. H., International Publishers, New York (1961).

³⁶ Skinner, B. F., "The Behavior of Organisms: An Experimental Analysis," Appleton Press, New York (1938).

Skinner, B. F., "Squirrels and Human Behavior," Maxwell-Ham, New York (1946).

³⁷ Skinner, B. F., "Contingencies of Reinforcement: A Theoretical Analysis," Appleton-Century-Crofts, New York (1969).

Skinner, B. F., "Cumulative Record," 3rd ed., Appleton-Century-Crofts, New York (1972).

³⁸ Skinner, B. F., "About Behaviorism," Knopf, New York (1974).

³⁹ Skinner, B. F., "The Behavior of Organisms: An Experimental Analysis," Appleton Press, New York (1938).

Operant conditioning is exemplified by one of the experiments of Thorndike where a dog was trained to escape from a "puzzle box" in order to obtain a morsel of food and the companionship of his fellow laboratory animals. In order to escape, and thus achieve its reward, the animal was required to perform some act—an operant—such as pressing a lever, pulling a cord, or merely pushing a door. An operant then may be defined as a conditioned reaction to an environmental stimulus.

Operant reactions which have been repeated many times ultimately may be performed without conscious thought. The simple act of unlocking a door while talking or the act of driving an automobile over a familiar route while pondering some problem are excellent examples of fully conditioned operant behavior.

As the neural pathways which control the operant responses become firmly established, the threshold for eliciting the operant behavior is reduced greatly over that existing in the initial trials. Thus the ease of driving a vehicle over the familiar pathways to and from work becomes less and less demanding as a conscious entity, as every commuter knows. The threshold of stimuli drops to such a level that the total stimulus input may not reach the conscious level at all times, as evidenced by the oft heard statement, "I don't really remember the drive home, because I was too preoccupied with" The same phenomenon must also apply to the test subjects of this experiment, but there was no attempt to quantify the minimum level of olfactory stimulus which would evoke the desired behavior in experienced rats.

The work of Pavlov, which originated in his experiments in digestive processes, elucidated an entirely different training process which required nothing of the animal but its conscious state. A familiar example of this form of conditioning is the response of a hungry animal to the sight or smell of food—i.e., it salivates in preparation for ingestion of the food.

A question now arises as to which of these forms of the learning experience is the more important to the animal so conditioned. If one considers the case of a so-called wild animal existing in its natural habitat, then operant conditioning is the more important, since this form of learning will probably determine the survival of the animal. The most common example of this premise is the search for food. Any wild animal which has survived infancy has been conditioned to react to the sight or smell of a food target by a series of motions (operants) which will deliver food to the mouth. In anticipation of this event (consider a cat about to pounce on a mouse), intensive salivation occurs which readies the oropharynx for the first steps of ingestion of the food. This salivation is a prime example of classical conditioning.

In this particular wildlife scenario, the inescapable conclusion is that operant conditioning is vital to survival, whereas the classical conditioning serves basically as a convenience in the acceptance of the first food morsel, and thereafter this learning paradigm is of secondary significance. As Wyrwicka¹² states, "It seems plausible that in nature classical reactions serve to get the animal ready, and thus facilitate the performance of instrumental reactions."

This theorem is exemplified in humans by the autonomic response to a fight-or-flight situation, such as the approach of a singularly unfriendly dog during an otherwise peaceful repose in a relaxed environment. An infant may exhibit little or no response to the snarling animal since the infant has not been conditioned to expect a personal assault from the evidence presented by the sight and sound of the hostile dog, while an adult who has learned of the potential consequences of such an encounter is faced with the option of fighting off the dog or of running away. In the adult case, classical conditioning evokes a reaction in the sympathetic nervous system which releases catecholamines, dilates the pupils, increases skeletal muscle blood flow, and so on, and this response, in turn, facilitates whichever operant response follows the encounter.

Thus, in situations where the survival of the individual is at stake, certainly operant conditioning is the dominant training protocol. But situations do arise where classical conditioning is of great value to the individual. Of the many examples possible, only that presented by the instant research will be explored.

Consider that:

- a. An animal has been trained to associate a sensory stimulus with the opportunity to achieve (by an operant) an extraordinarily profound sensation, such as electrical brain stimulus (EBS), which transcends any normal desires the animal has experienced to date and
- b. The previous opportunity to effect self-stimulation has been removed and only the sensory stimulus remains, and
- c. The ultra-desirable EBS-induced sensation will appear *only if* the animal is capable of manifesting a certain brain wave (EEG) pattern immediately following the sensory stimulus.

¹² Wyrwicka, W., *The Mechanisms of Conditioned Behavior: A Critical Look at the Phenomena of Conditioning*, U. of Illinois Press, Springfield, Illinois (1972).

Should this type of EEG arousal occur, the effect is a classical reaction by definition, since no overt act on the part of the animal has taken place. The presence of an EEG change which is indicative of an anticipation of the impending reward would most probably be a carry-over reaction from the operant paradigm where the anticipatory pattern caused the animal to effect the operant which would bring about the reward. Since the presence of the classical conditioning reaction would of itself be sufficient to elicit the desired reward, the classical reaction would then be reinforced and would become increasingly more evident. At this point, the heretofore involuntary classical response could be viewed as voluntary, and one may conclude that conscious control of autonomic function has occurred.

Certainly, there is ample evidence in the literature dealing with biofeedback experiments that both classical and operant conditioning can effect autonomic responses. The important point here is that while classical conditioning may not be a survival factor for the test subject, it assuredly has a tremendous behavioral impact in certain situations where a desired result is to be obtained without voluntary physical action on the part of the subject.

This response to conditioning exemplifies the oft stated concept that every adult functioning individual is a creature whose conditioning is appropriate for its survival, and this conditioning is a complex mixture of classical and operant paradigms induced by the environment in which survival must occur. Many behavioral scientists profess that the environment, as interpreted by the genetic makeup of the individual, affects that individual to such an extent that it may ultimately be powerless to shape its own destiny. While this may not be completely true in many instances—as with man—it certainly appeared to be true in the case of the subjects of this experiment, which seemed to be completely unable to react properly for survival when given the option of indefinitely achieving the reward of brain electrostimulus.

9. A Choice of Stimulus. In a program designed to use a living organism as a bio-sensor, the experimenter, having chosen the test subjects and the general conditioning paradigm, must ultimately select the reinforcement stimulus which is most appropriate to the research effort.

Initially, a decision must be made as to whether to use rewarding or aversive stimuli, or whether some mix of these two basic forms may be best. There are sound arguments in favor of each of these antagonist forms of reinforcing stimuli, but, at the conclusion of extensive reading from the bibliography materials, and after numerous discussions with persons actively experimenting with rats, it was determined that there would be no experimental advantage accruing from the use of any form of aversive stimulus.

The number of possible rewarding stimuli are few. Food, water, and electrical stimulation of the brain clearly emerged as the only feasible techniques. Much has been written of the use of food and water rewards in the case of rats, and certainly these rewards are simple to implement in comparison to electrical brain stimulation. However, there are certain basic disadvantages to the use of sustentative rewards. First, the animal must be maintained in a state of deprivation for a considerable period prior to the actual experimental work if this reward (water or food) is to have maximal effect as a reinforcing stimulus, and this deprivation is not without adverse physiological sequelae, which may be severe enough to alter the sensorium and/or the normal emotional status of the subject, thereby biasing the experimental results in an uncertain manner and to an indeterminate degree. Further, as the experiment progresses, the previously deprived animal gradually will achieve some degree of satiation, and thereby the effectivity of the nutritional reward is diminished by an unpredictable amount. Likewise, as normal physiological parameters are restored from the deprived state, there certainly will be alterations in behavior which are, again, indefinable.

EBS is quite another matter. It is difficult to implement since delicate surgery is required, and there is a continuing risk of irreparable damage to the subject should the stimulus electrode become dislodged. But these disadvantages are far outweighed by the resulting stability of performance and the intensity of stimulation.

Since there is no physiological or emotional deprivation of any kind either necessary or desirable with EBS, the animal may always be maintained in a normal state of homeostasis with the consequence that there is minimum variance in its sensory and emotional performance from hour to hour and from day to day. Further, and most importantly, the degree of stimulation attainable by EBS is significantly more pronounced than that obtained with nutritional rewards, and the overall effect is—as observed both during this experiment and during many others reported in the literature—an essentially constant response to the reinforcement stimulus, regardless of the period of stimulation. This phenomenal constancy was observed even when homeostasis failed due to self-induced deprivation and extreme fatigue.

There are references in the bibliography which show that in all of the infra-human mammalian species tested to date, there are one or more areas in the brain where an electrical stimulus will evoke the apparent sensation of extreme pleasure, just as there are areas where electrostimulus will evoke fear, rage, complacency, hunger, anorexia, and so on.

James Olds,¹⁹⁷⁹ a pioneer in the area of electrical stimulation of the brain, published a series of papers in the mid-1950s, which dealt specifically with the role of brain stimulation in behavior modification in rats.

A study of the works of Olds and others leads to the conclusion (and one must admit a degree of subjectivity in the interpretation of these works) that electrical stimulation of a certain amplitude and duration applied to specific regions of the limbic system of most infra-human mammals results in an emotional state—perhaps it is pleasure or perhaps it is undefinable—which transcends all normally evoked emotional states.

This phenomenon is quite pronounced in the rat, as evidenced by the repeated observation by many highly qualified research workers. Olds and others have repeatedly demonstrated that rats will endure great physical distress (e.g., severe foot shock, high noise levels) to achieve self-stimulus, and there are documented reports of animals which have died of dehydration to achieve self-stimulus, even though ample water supplies are

13. Olds, J. "Physiological Mechanisms of Reward." In Jones, M. R. (ed.) *Nebraska Symposium on Motivation*. University of Nebraska Press, Lincoln (1955).
14. Olds, J. "Pleasure Centers in the Brain." *Scientific American* 193:103-106 (1956).
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22. Olds, J. "Differential Effects of Presses and Levers on Self-stimulation of Different Brain Sites." In *Electrical Stimulation of the Brain*. University of Texas Press, Austin (1960) (1961).
23. Olds, J. "Hypothalamic Substrate of Reward." *Physiological Review* 42 (1962).
24. Olds, J. In: Aikay, W. R. and Takazumi, T. (eds.) *Progress in Brain Research* 27: Structure and Function of the Limbic System. Elsevier Publishing Co., New York (1963).
25. Olds, J. and Milner, P. "Positive Reinforcement Produced by Electrical Stimulation of Septal Area and Other Regions of Rat Brain." *Journal of Comparative and Physiological Psychology* 17:119-27 (1954).
26. Olds, J. and Old, M. E. "The Mechanisms of Voluntary Behavior." In: *The Role of Pleasure in Behavior*. Hoeber Medical Division, Harper and Row, New York (1963).
27. Olds, J. and Old, M. E. "Approach-Avoidance Analysis of Rat Decision-Making." *Journal of Comparative Neurology* 120:212-229 (1963).
28. Olds, M. E. and Old, J. "Approach-avoidance Interactions in Rat Brain." *American Journal of Physiology* 203:1903-10 (1962).
29. Old, M. E. and Olds, J. "Effects of Lesions in Medial Forebrain Bundle on Self-stimulation Behavior." *American Journal of Physiology* 211:1253-1261 (1962).

just beyond reach of the stimulus treadle. Most researchers agree that as a rule, nothing short of disablement or loss of consciousness will deter a rat from its attempts at EBS self-stimulation when such opportunity is present.

It is conceivable to humans that this remarkable result of limbic stimulation can be in any manner aversive; therefore, one may argue that it must be an "ultimate pleasure" and for this reason, the region of the brain where electrostimulus can produce this effect is called the "pleasure center."⁶⁰ Morphologically, this area varies somewhat from species to species, but in general the effect is observed to occur with stimulation in the general area of the lateral hypothalamus.

It was unnecessary to expend any experimental effort toward locating the optimum site for EBS, since the work of Olds and Milner⁶¹ in 1954 determined the site for the most effective positive reinforcement in rats to be the Medial Forebrain Bundle (MFB). (See Figure 1.) Olds and Olds⁶² clarified the location by stating that "... electric shock applied to points in the Posterior Medial Forebrain Bundle (MFB) regions of the lateral hypothalamus produced very high rates of responding at very low levels of stimulation ... it seemed that maximum effects (highest rates of self-stimulation responding) could be obtained in this posterior hypothalamic region with minimal levels of stimulation as compared with effects obtained elsewhere in the central nervous system."

A contrary view was taken by Valenstein and Campbell⁶³ who argued that extreme lesions induced in the MFB did not lessen self-stimulation, and that septal stimulation was a more effective positive-reinforcement EBS. Wilkinson and Peale⁶⁴ demonstrated that EBS introduced into 19 sites in the rat produced a positive reinforcement response, with the best results observed from stimulation in the Posterior Lateral Hypothalamus. Other authors cite successful positive reinforcement in various sites in the mammalian limbic system, but the general consensus appears to favor the Posterior Medial Forebrain Bundle as optimal. It is interesting to note that, while there is some disagreement among researchers as to the location of the optimum pleasure center site, no reference could be found which referred to the MFB as a site of possible negative reinforcement (aversive stimulus) unless the stimulating currents were quite high. In this latter case, one would suspect that high current stimulation would result in significant stimulus currents reaching areas such as the medial geniculates and other thalamic nuclei, which have been shown to generate negative reinforcement.

⁶⁰ Olds, J., "Pleasure Centers in the Brain," *Scientific American* 195:106-116 (1956).

⁶¹ Olds, J. and Milner, P., "Positive Reinforcement Produced by Electrical Stimulation of Septal Area and Other Regions of Rat Brain," *Journal of Comparative and Physiological Psychology*, 47:419-27 (1954).

⁶² Olds, M. E. and Olds, J., "Effects of Lesions in Medial Forebrain Bundle on Self-stimulation Behavior," *American Journal of Physiology* 217:1253-61 (1969).

⁶³ Valenstein, E. S. and Campbell, J. E., "Medial Forebrain Bundle-Lateral Hypothalamic Area and Reinforcing Brain Stimulation," *American Journal of Physiology* 210:270-1 (1966).

⁶⁴ Wilkinson, H. A. and Peale, L., "Intracranial Self-stimulation in Cats," *Journal of Comparative and Physiological Psychology* 121:435-40 (1963).

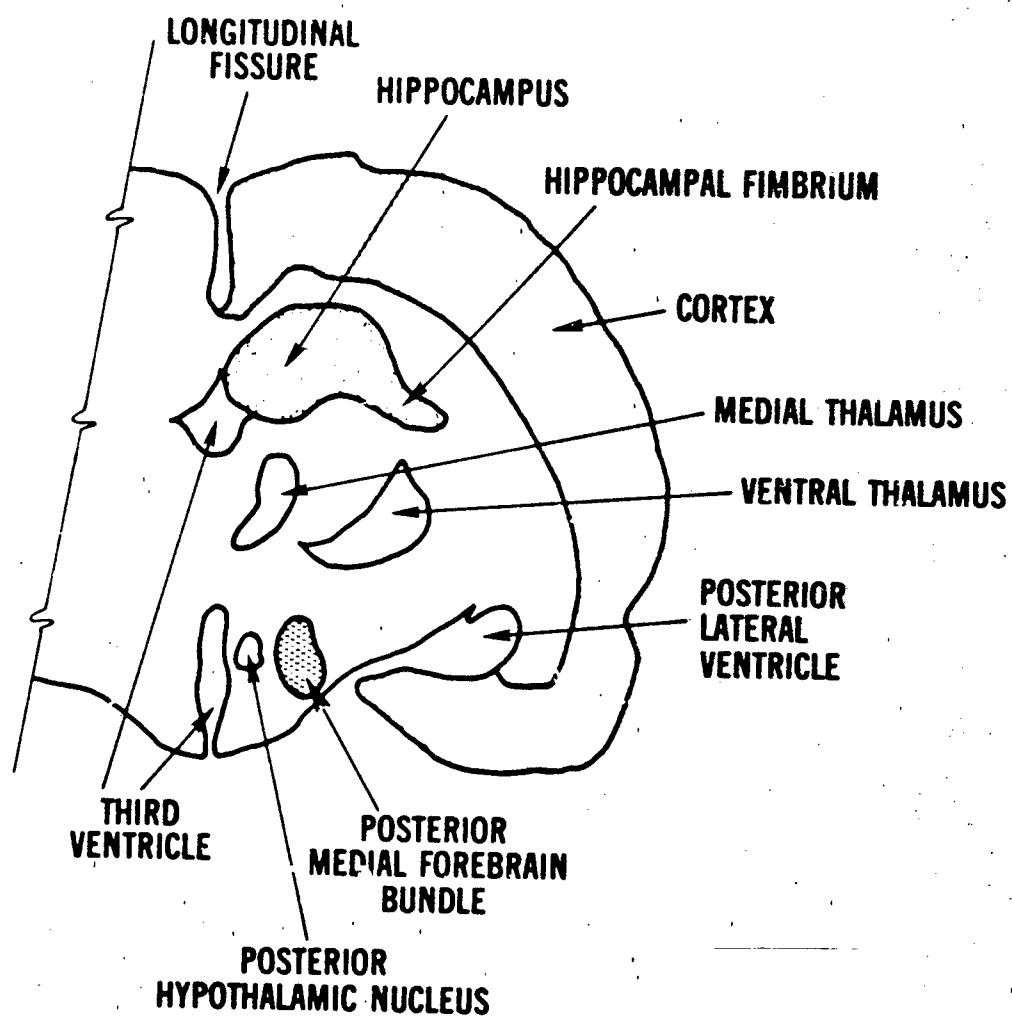


Figure 1. MFB and landmark structures in the vertical stereotaxic plane.

Since the least potential for physiological damage occurs at the lowest stimulating currents, and since the MFB seemed to accept the greatest latitude of stimulating currents, this site was chosen for all subjects used in the experiment under discussion. This acceptance latitude possibly permitted successful implementation of the EBS electrode in a greater number of subjects than one would expect with other, more critical sites, and this was a significant consideration, since some errors of implant were bound to occur even though the subjects manifested a high degree of unit-to-unit structural agreement.

The validity of this belief would appear to be verified by the fact that better than 90 percent of all implants resulted in an acceptable level of positive reinforcement with no evidence of any aversive responses, although there were two instances where a myoclonic seizure appeared with application of EBS. The latter cases were believed to result from damage to the motor cortex or the internal capsule which occurred during implant surgery.

Since the majority of known data on the effects of brain electrostimulus in humans is derived from the adventitious application of such stimulation during brain surgery, it is not surprising that relatively little is known of its effects on the limbic system of man. Work is proceeding slowly in this area for obvious reasons, and the current limitations of information preclude extensive comparisons of the effects of animal brain electrostimulus with the human experience, but in the light of all available evidence, one must conclude that there is no human correlate to the emotional effects which brain electrostimulus generates in the pleasure center of the so-called lower animals.⁶⁵ It is pointless, then, to attempt to understand the nature of the emotion resulting from the particular conditioning stimulus used in this series of experiments, but the evidence of this research indicates that EBS represents the most effective behavioral conditioning stimulus for infra-human mammals yet encountered.

10. Concepts of Electroencephalography. This experiment utilized the neural signals normally identified as electroencephalogram signals, but this discussion will not extensively embrace current theories as to the nature of these signals since there is so little known in this area that a major discussion would amount to mere speculation (as was the case with olfaction). Thus only the most basic aspects of the subject are treated here.

⁶⁵ Heath ("The Role of Pleasure in Behavior") reported that stimulation in the septal region of human subjects resulted in pleasant sensations, a feeling of being comfortable and a desire for repeated stimulations. This author also noted that stimulation of the rostral hypothalamus resulted in anxiety, while stimulation of the caudal diencephalon, the rostral mesencephalic tegmentum, and the rostral hippocampus, resulted in stages of extreme fear or rage.

There is little agreement among electroencephalographers as to the source of the surface potentials which comprise the EEG, and there is no agreement on the location of (or even the existence of) a central EEG pacemaker. It is certain that the EEG electrodes used in this experiment picked up the summed activity of a great number of neurons (there are about 5×10^5 neurons per square millimeter of cortical surface) at various depths.

Of the many theories which attempt to explain the signals, two appear to dominate the literature. One concept claims that the apical dendrites and axons of cortical neurons could not contribute significantly to the EEG since the small diameters of these structures would preclude large ion currents in the extracellular fluids, and hence the dipoles formed would be weak signal sources. However, according to this theory, the action potentials generated on the soma at the axon hillock are capable of large ion current release. The soma spike is small, however, and EEGs have been recorded when anesthesia precluded cortical axon firing.

The other dominant theory is essentially the converse, and claims that apical dendrites of the pyramidal cells are the basic source of the EEG. Since most of the pyramidal cell bodies lie in cortical layers III and V, the resulting dendrites are long and could be effective dipoles. Some research teams claim that the EEG is the result of postsynaptic potentials, both excitatory and inhibitory, developed by the pyramidal cell soma and dendritic projections, and there is some evidence obtained from microelectrode comparisons of cortical cell signals with surface wave activity which tends to support this latter concept.

The existence of a pacemaker which might at least tend to synchronize the Excitatory Post Synaptic Potential (EPSP) or Inhibitory Post Synaptic Potential (IPSP) of the pyramidal cells has not been established unequivocally. Some evidence exists that would indicate spontaneous cortical rhythms, while other evidence argues for an external source somewhere above the rostral end of the brain stem. The thalamus has been suggested in this latter instance as a likely locus, but this rationale is largely based on sheer speculation.

Thus, little of significance to the program was gained from a rather extensive review of the literature of electroencephalography. It is not possible to suggest a source for an AESC in the light of current knowledge and it is not reasonable to presume that further thought could suggest a more optimal approach than that taken in this research, since nearly all knowledge of the nature of the EEG is too speculative to be considered in the category of scientific fact.

11. EEG Electrode Sites. Since one of the objectives of this experiment was the elucidation of an anticipatory evoked spectral change in the EEG of the conditioned subjects, it is apparent that one of the more important preliminary considerations was the location of suitable EEG electrode sites. It was evident that the best system signal-to-noise ratio could be attained by use of direct indwelling cortical electrodes at appropriate sites, but this solution necessarily implied that suitable locations were known a priori. Obviously, this was not the situation, and thus the use of cortical microelectrodes was contraindicated because of the very small area of neural tissue which would thus be supplying EEG signals.⁶⁶ Gross indwelling cortical electrodes with an area of 1 mm² or more were excluded because of mechanical problems, such as cortical abrasion or avulsion, which would cause widespread necrosis, with consequent signal loss and possible brain dysfunction.

After due consideration, it was determined that, while gross electrodes were best for the purpose, a supra-cortical location would be ideal, since this choice would allow for some limited diffusion of the cortical EEG signals, resulting in cortical signal coverage over several square millimeters, and it would greatly facilitate the implant procedure, while at the same time, greatly reducing the risk of trauma and infection. Consequently, an epidural location was decided upon and only the choice of appropriate site coordinates remained.

It appeared that a parietal location might be significant, since an electrode at this site could be positioned to detect signals originating in the central association areas posterior to the central fissure. If the anticipatory event was a prelude to a motor event, then this location might evidence the highest signal level, although the diffuse nature of associative signals was known to be a problem from preliminary experiments.

At least one electrode responsive to limbic system activity was desirable, but these areas lie generally well below the cortex (about 6 to 8 mm in the rat). The best location was determined to be directly above the cingulate gyrus and as near to the sagittal fissure as possible. An electrode so positioned would assuredly pick up signal originating in the frontal lobes, as well as from the motor areas anterior to the central fissure, and, unfortunately, it would pick up muscle artifacts resulting from eye movements and from motions of the nares occurring during sniffing. Nevertheless, in a brain as small as that of the rat, there are only a few choices of electrode location, and the electrode referred to hereafter as the cingulate electrode was placed in the closest site attainable with simple surgery.

⁶⁶ This small area of signal collection would require perhaps hundreds of trials using thousands of subjects in order to demonstrate the optimum location. Such a task was beyond the scope of the research.

After determination of the two principal sites, it was determined that one electrode location was feasible in the occipital area even though this zone was thought to be a somewhat unlikely location for limbic signals to be detected. In all honesty, this electrode was included in the experiment because there was space available at no appreciable added risk to the subject and since there was a remote possibility of some serendipitous result. The final electrode locations are shown in the sketch of Figure 2. The appropriate brain structures served by the three EEG electrodes are depicted in Figure 3.

The location of the indifferent electrode evoked considerable thought since any location is less than ideal. Muscle artifacts can generate serious EEG anomalies and greatly upset signal data analysis so one must choose a location as free of large muscle action potential signals as possible. The electrode must be sufficiently distant from the signal electrodes so that the return pathway is not proximal to the signal pathways. Further, the electrode site must be structurally capable of accepting the electrode with minimal danger of accidental dislodgement, and certainly the site must be such that the electrode poses no threat to the survival of the subject in the event of electrode motion. Considering all factors, the nasion was chosen as optimum, or more realistically, as the lesser of all evils.

II. METHODS AND MATERIALS

12. Stimulus and EEG Electrodes. A survey of available devices evidenced the fact that many of the commercially available electrodes were unsuitable for chronic brain implant service since either the materials used were toxic to neural tissues or the electrode dissolved so rapidly that the implant would not survive to the service life expectancy of the biosensor animal. Ultimately, the choice was between platinum and stainless steel.⁶⁷ Stainless steel was selected in spite of its rather high drift voltage because it was available in many standard forms, because it was relatively inexpensive, and because it is known to cause little tissue necrosis in chronic implants.

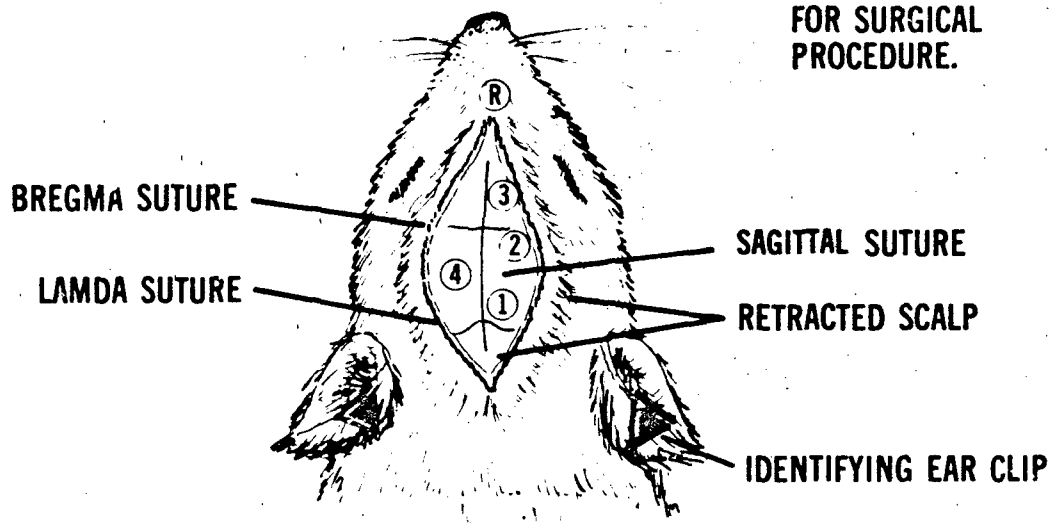
Delgado⁶⁸ reported that, after several months of chronic electrode implant, necropsy disclosed that a thin glial capsule had surrounded the stainless steel electrode, but that there was no evidence of local excitability except at the exposed end of the electrode. Further, there was no local or general irritation and no neural degradation beyond the direct path of the electrode. Cooper and Upton⁶⁹ noted that in human cases of chronic cerebellar stimulation for periods up to 5 years, there was no clinically evident disturbance or significant tissue damage.

⁶⁷ Even platinum (191) and stainless steel will eventually dissolve, especially if stimulus currents exceed 10 milliamperes.

⁶⁸ Delgado, J. M. R., "Therapeutic Programmed Stimulation of the Brain in Man," In: Sweet, W. H. (ed) *Neurosurgical Treatment in Psychiatry, Pain, and Epilepsy*, University Park Press, Baltimore (1977).

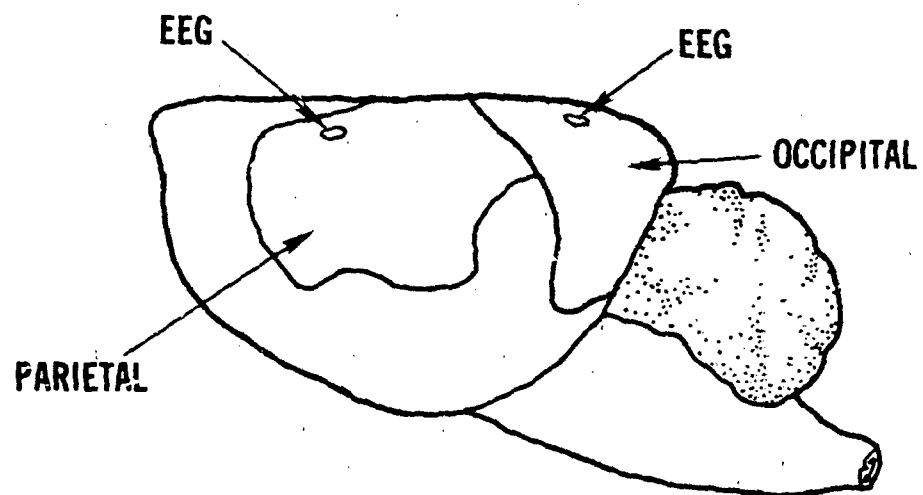
⁶⁹ Cooper, I. S. and Upton, A. R. M., "Use of Chronic Cerebellar Stimulation for Disorders of Disinhibition," *Lancet* 595:680 (1978).

**NOTE: SCALP IS RETRACTED
FOR SURGICAL
PROCEDURE.**

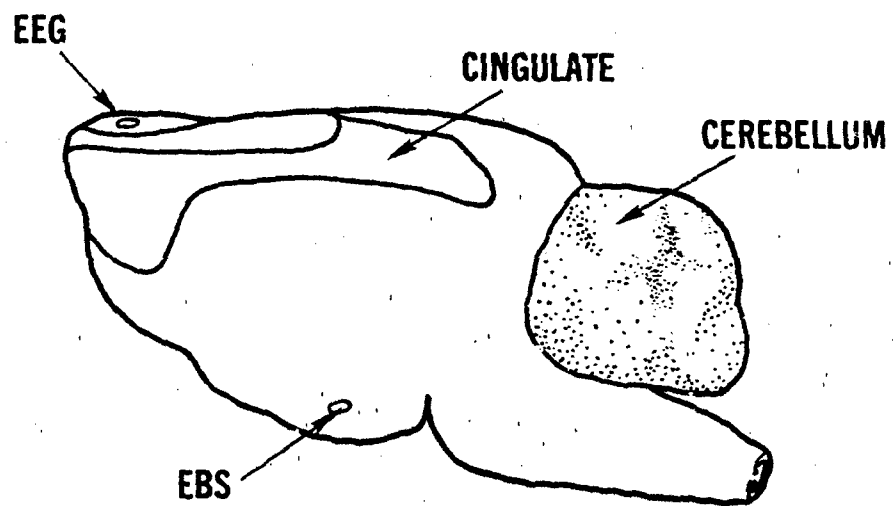


- 1 = OCCIPITAL ZONE (EEG)**
- 2 = PARIETAL LOBE (EEG)**
- 3 = CINGULATE GYRUS (EEG)**
- 4 = MEDIAL FOREBRAIN BUNDLE (EBS)**
- R = NASION (INDIFFERENT ELECTRODE)**

Figure 2. EEG and EBS electrode sites (trephine locations).



PARIETAL VIEW



SAGITTAL VIEW

Figure 3. Parietal and Sagittal views of EEG and EBS electrode sites.

The specific stimulus (EBS) electrode chosen was a Teflon-insulated, 10-mil diameter twisted pair manufactured by Plastic Products, Inc., which had performed well in the experiments of other researchers who employed chronic indwelling brain electrodes in rats. Throughout this research program, there was no evidence of physiological deficit resulting from the proper use of this electrode.

The EBS electrode, the indifferent electrode, and the EEG electrodes were connected to the appropriate instrumentation through the so-called skullcap connector. This connector was not all that one might desire, since it utilized a threaded mating connector which often required extreme torque for removal. However, of all available connectors surveyed, this was the most rugged and noise free, and hundreds of hours of use attested to its reliability.

Motion of the EBS electrode can occur should the electrode-skullcap connector abduct from the skull; therefore, extreme care was taken during surgery to assure a firm bonding of this connector to the skull (see paragraph 15, "Surgical Procedures"). In those instances where the cap loosened, there ensued severe brain trauma, usually resulting in epileptic seizure, often with accompanying ataxia and bizarre behavior. These subjects were euthanized since early experiments evidenced permanent neurological damage with no hope of a successful contralateral implant. Fortunately, most animals tolerated the skullcap well but a few attempted to dislodge the cap at every opportunity, and while two were successful in this action, at least five were lost simply due to the severe torque generated in removing the mating signal connector.

Since there was no a priori knowledge of any specific cell cluster which might be a likely source of the anticipatory spectral change, the use of microelectrodes (or even the 10-mil stimulus electrodes) as EEG pickup electrodes was contraindicated. As noted earlier, a large electrode in direct contact with cortical tissue was deemed inadvisable, and thus it was determined that an electrode with a contact area of 1 mm² or better, placed on the surface of the dura mater would serve admirably. For this purpose, an electrode was devised which consisted of a stainless steel 0-80 screw welded to a stainless steel wire (10-mil diameter) to which a mating contact from the electrode cap was also welded. As described under "Surgical Procedures," these screw electrodes were threaded, by self-tapping action, into the skull proximal to the cortical areas of interest, to a depth sufficient to firmly contact the dura mater.

The indifferent electrode was a 5-mm stainless steel rod welded to 10-mil diameter stainless steel wire, which in turn was welded to the cap contact. The wire used between all electrodes and the skullcap was teflon coated to preclude extraneous signal pickup in the physiological media present under the scalp.

13. Muscle Artifacts in EEG Data. Preliminary experiments with a few subjects soon demonstrated the need for a method to reduce the number and magnitude of muscle artifacts to a minimum. The idea of inducing muscle paralysis by the use of some neurotoxin such as the curariform drugs was rejected as a solution to the artifact problem, since there was no known drug which would selectively inhibit cholinergic synaptic transmission to most skeletal muscle while uniquely sparing intercostal and diaphragm neuromuscular activity. Thus, the use of curariform agents would have required the use of a respirator, which was totally inimical to the overall goals of the experiment.

After several trials, a restraint device (see Figure 4) was devised which consisted of a tightly fitted hemicylinder which was placed over the subject (usually after a bit of preliminary struggle) which became nearly motionless after it adapted to total restraint, and remained thus for a half hour or more at a time.⁷⁰ There was some motion of the head during periods of non-stimulation, and some occasional furious sniffing during odorant runs, but in general the restraint was quite successful in reducing motion artifacts.

An additional benefit was obtained from this method of restraint in that the subject was always oriented such that its head was directly in the stream of incoming air, and thus maximum availability of incoming odorants was assured at all times. The simple restraint was a valuable aid in simplifying the task of signal data (EEG) reduction, and future detection systems employing rats will unquestionably use an even more effective restraint system.

14. EBS Signals. The waveform used for EBS must be structured carefully so that there is:

- a. No aversive response to stimulation.
- b. No physiological deterioration after many thousands of stimulations. A problem of aversive reaction to electrostimulus can result from two causes:
 - (1) The electrode is incorrectly positioned, or
 - (2) The signal amplitude or duration (or both) is excessive.

⁷⁰ It was not surprising that the subjects adapted so readily to total restraint, since rats are by nature thigmotactic and they apparently derive comfort from a tight fitting environment. In addition, those animals with EBS experience were aware that delivery of highly desirable stimulus could result from their containment, and there was usually no struggling against the restraint after delivery of the first EBS, unless the periods between EBS events exceeded 30 minutes.

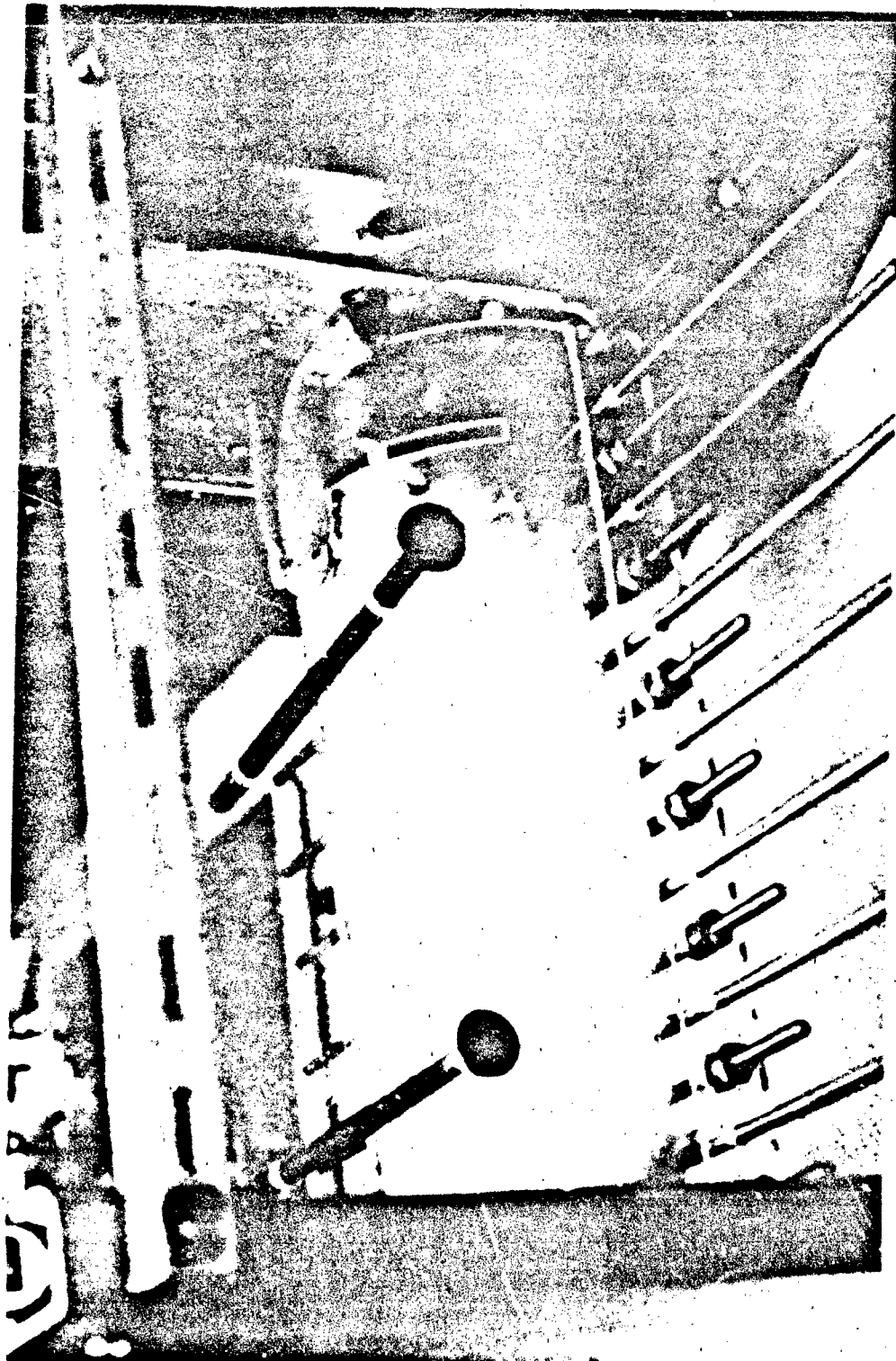


Figure 4. Test subject in restraint device.

Physiological deterioration can result from:

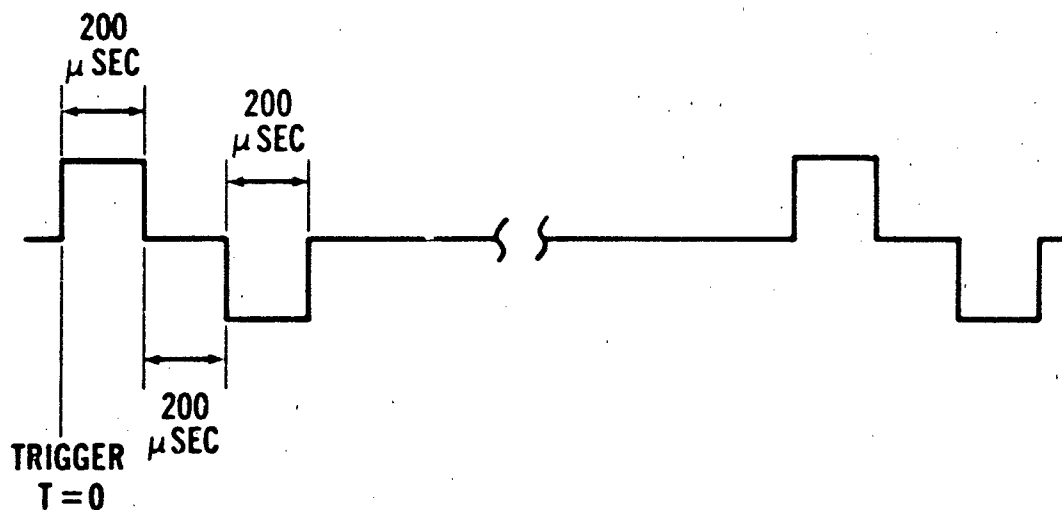
(3) Electrophoresis (with resultant proximal tissue necrosis) due to the stimulating current, or

(4) Motion of the electrode resulting in widespread trauma and consequent gross tissue necrosis.

When initial stimulation resulted in aversive response, the signal parameters were manually varied as the subject was observed. If no combination of signal parameters (a normal range was soon obtained in this research) would elicit the pleasure response, then obviously the implant was incorrect and the subject was of no experimental value. Figure 5 shows those signal parameters determined to be optimum for this research.

The signal was bipolar in order to satisfy requirement 1Ba3), above, since continuous monopolar stimulation is generally acknowledged to cause cellular necrosis adjacent to the electrode by the action of electrophoresis. In order to prevent local necrosis due simply to the heating effects of the stimulating current, the waveform generator was a constant-current device. Experience has shown that this device protects the subject, while adequately stimulating the MFB. This statement is verified by the fact that more than 24 subjects were allowed to self-stimulate for periods totaling 40 h each during conditioning runs with no evident physiological deterioration, and since the average subject self-stimulated initially at a rate of nearly 2/s, and generally demonstrated average rates in excess of 1000 stimulations per hour for up to 4 h, any deleterious physiological effects would have been readily apparent long before the conditioning period had ended. Overall, there was no evidence of diminution of self-stimulus in test subjects regardless of the number of hours occupied in conditioning self-stimulation and in the subsequent data runs. Neither was there evidence of any type of aberrant behavior which would indicate physiological damage (except for the aforementioned cases where the EBS electrode actually moved).

It is pertinent to note that all animals placed in the "acceptable" category responded to average stimulus currents in the range of 100 μ A to 500 μ A. No subject would respond reliably to EBS levels of less than 100 μ A, and the upper limit of 500 μ A was set for two reasons. First, there was some concern for heating damage based upon histological data from other experiments, and second, it was found in limited trials that subjects requiring currents in excess of this upper limit tended to manifest symptoms of widely diffused stimulation which included seizures and motor disturbances.



The signal generator possessed the following capabilities:

Output: constant-current-baseline clamp to 0 V d.c.*

Output current: 10 to 10,000 μA continuously variable.

Pulse duration: 50 to 1,500 μs continuously variable.

Pulse rise time: $< 17 \mu s$.

Pulse group repetition rate: zero (triggered) to 200 Hz continuously variable from 5 Hz.

During the data runs, the following parameters were held constant:

Pulse group repetition rate: 100 Hz

Pulse groups per stimulation event: 20 pairs.

Stimulus current: 300 μA (average per animal).

* The baseline clamp was made adjustable to compensate for small currents generated at the electrode/tissue interface in order to minimize electrophoresis.

Figure 5. EBS signal waveform.

All of the test subjects used in the final data runs were stimulated at 300 μ A regardless of their measured response threshold. This was done so that some idea of the expected performance of a "mass-produced biosensor rat" might be attained. There was no indication that this stimulus level was other than optimum for this experiment.

15. Surgical Procedures. The sites for electrode implant have been discussed in detail. Briefly, cortical EEG electrodes were juxtaposed to the dura mater proximal to the following general areas: occipital lobe, parietal lobe, and cingulate gyrus.

Since the cortical EEG electrodes were large area electrodes and since the detected signals were somewhat diffuse due to the nature of the meningeal membrane, the exact location of these electrodes were not considered to be critical.

The location of the EBS electrode, however, was quite critical, since that electrode had to terminate in a small cell cluster (the MFB; specifically, the baso-medial area near the level of the Islands of Calleja). (See Figure 1 and paragraph 9, "A Choice of Stimulus.")

The test subjects were selected to have body weights ranging between 250 g and 350 g. These limits were selected since body weight is a good analog of body size in this strain of rats, and body size must be controlled if the stimulus electrode is to be accurately implanted by use of a specific stereotaxic atlas. Even with these size limits, not all animals thus implanted were useful to the experiment, since some variations in brain geometry occur from subject to subject in any population no matter how great the apparent structural similarities.

Electrode implant was accomplished by use of a David Kopf Model 900 Small Animal Stereotaxic Apparatus (see Figure 6). The subject was prepared for surgery by a two-step anesthetic procedure. First, a parenteral (abdominal muscle) administration of 1 cc of Chloropent (active ingredients: chloral hydrate and pentobarbital) rendered the animal essentially motionless.⁷¹ The subject was then weighed and a second injection of Chloropent administered in accordance with Table 2. When the animal exhibited no response to strong squeezing pressure on its paws and did not manifest a blink reflex to gentle air puffs directed into its eyes, it was known to be sufficiently anesthetized to begin implant surgery. When necessary, a maintenance injection of 0.2 cc of Chloropent was given at 30- to 45-min intervals.

⁷¹ There are many regimens for anesthetizing laboratory animals, some of which use IM or subcutaneous administration of a substance such as Ketosec (ketamine HCl) or Surital (thiamylal sodium) for induction, followed by a gas, such as Halothane or Metathane, for maintenance. Since it was inadvisable to subject laboratory personnel to long-term exposure to gas anesthetics, Chloropent was used, even though it presented a risk of inducing respiratory paralysis in the test subjects.

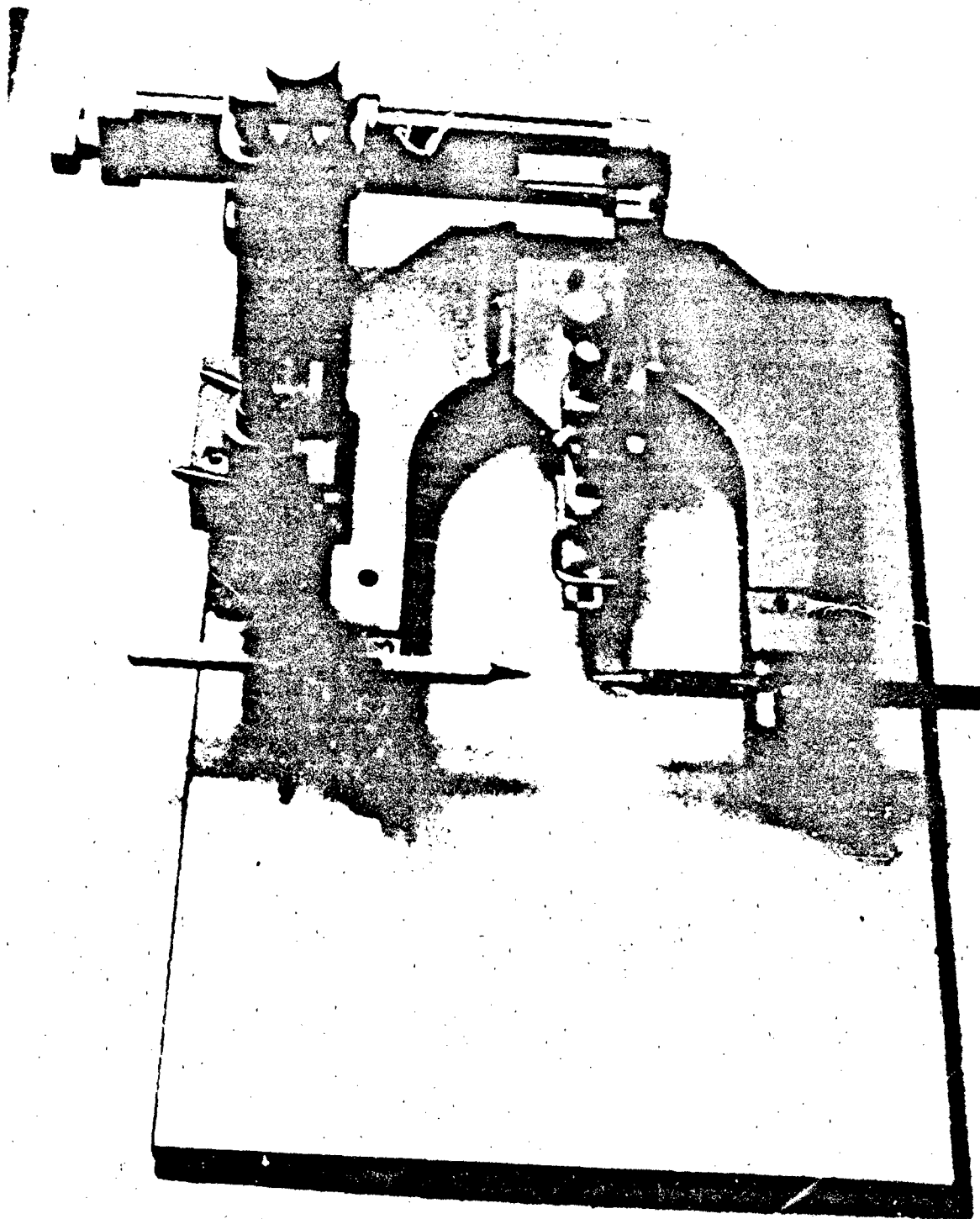


Figure 6. Small animal stereotaxic apparatus.

Table 2. Chloropent Anesthesia Dosage

Body Weight (g)	Chloropent (cc)
250	0.75
300	0.88
350	1.00
400	1.13
450	1.29
500	1.45
550	1.61
600	1.71

Note: A dosage of twice the above amount may induce respiratory distress in some subjects. LD₅₀ is at least three times the listed amount.

Chloropent ingredients: Mg SO₄, propylene glycol, chloral hydrate pentobarbital

The anesthetized subject was placed prone in the stereotaxic apparatus and a mouth restraint was inserted. Next, ear bars were inserted full depth into the auditory meatus to render the head essentially immobile in all planes (see Figure 7). After a final check of the depth of anesthesia, the scalp was shaved from nasion to occiput and from ear to ear, and the area was thoroughly disinfected with alcohol. The eyes were then lubricated with mineral oil to prevent drying of the conjunctiva (which would result from anesthesia-induced inhibition of the blink reflex).

Then a sagittal-plane scalp incision was made from the nasion to well past the Lambda suture, the scalp was retracted (employing suitable gauze dams to diminish bleeding) and the skull was scraped until all traces of the periosteum were removed. The entire area was then washed with alcohol and examined for minute traces of residual periosteum, since it was essential that the skull bone surface be absolutely free of this tissue in order that the electrode mount adhesive attain its maximum strength. Figure 2 shows the relative locations of all electrodes.



Figure 7. Test subject undergoing implant surgery.

The initial procedure in the development of the implant process was the determination of an anatomical coordinate system which could be used to define the electrode locations on all subjects. An inspection of the cranial surface of the subjects revealed the very distinct bregma (anterior), lambda (posterior), and sagittal (midline) sutures of this structure. From published stereotaxic data, it was evident that most researchers involved in rat brain experiments used some combination of the relative locations of these sutures as anatomical landmarks. Studies of rat brain morphology and cranial anatomy disclosed that there was high precision correlation between the location of the sutures and the medial forebrain bundle. The most common dimensioning techniques encountered in the literature used the arithmetic mean of the bregma-lambda distance plus a size-related correction factor to locate the anterior-posterior (A-P) location of the MFB. Similarly, the lateral position of the MFB was determined from the sagittal suture.⁷²

Since the Kopf apparatus did not have provision for setting any arbitrary point in any plane to zero, it was necessary to use the scale readings available to define the reference points. The procedure which finally evolved was as follows:

a. The sagittal plane was checked for coincidence with the lateral plane of the stereotaxic apparatus. There was a high degree of orthogonality of the sagittal suture relative to the plane of the ear bars in most subjects, so that the sagittal plane virtually coincided with the stereotaxic lateral plane when the subject was correctly positioned in the apparatus. Alignment errors of < 2 mm from bregma to lambda were generally not significant since the A-P dimension of the MFB was usually very close to the axis of lateral error and since small errors in EEG electrode placement were not of consequence.

b. Relative (scale) readings were found for bregma (B) and lambda (L) at the points of coincidence with the sagittal suture. The arithmetic mean of these readings $(B + L)/2$ were defined thereafter as A-P. The correction factor for animal size C, was found to be $(B - L)/0.765$. Combining A-P₀ and C gives A-P_c, the corrected anterior-posterior dimension. Lateral zero (S_0) was defined as the arithmetic mean of the lateral readings at the B and L intersections with the sagittal suture, or $(B_L + L_L)/2$. A lateral correction factor, K, of 1.5 mm added to S_0 was found to accurately define the lateral dimension (S_c) of the MFB.

⁷² In this series of experiments, the plane of the superior aspect of the skull was made as nearly horizontal as possible. Other experimenters use other attitudes for various reasons, and thus one cannot compare ERS electrode dimensions in other experiments without first determining the attitude of the skull in the stereotaxic device.

Thus:

$$A-P_o = (B + L)/2 \text{ mm}$$

$$C = (B - L)/0.765 \text{ mm}$$

$$A-P_E = (B + L)/2 + c/10 \text{ mm}$$

$$K = 1.5 \text{ mm}$$

$$S_o = (B_o + L_o)/2 \text{ mm}$$

$$S_E = (B_o + L_o)/2 + 1.5 \text{ mm}$$

where $A-P_E$ and S_E are the stereotaxic dimensions of the EBS electrode in terms of the unique scale readings for each subject. S_E was always located sinistrad to the sagittal suture.

c. The penetration depth, D_E , of the EBS electrode is as critical as the $A-P_E$ and S_E dimensions. Fortunately, the skull is somewhat planar in the space between bregma and lambda with small-amplitude cose striations. In the average rat, the optimum electrode penetration depth was determined to be 8.7 mm measured downward orthogonally from the mean surface height of the exterior skull at the point superior to the MFB. D_E was found as the arithmetic mean of the skull surface height $(B_o + L_o)/2$, where B_o was the skull height at bregma and L_o was the height at lambda where these sutures intersected the sagittal suture.

d. The cingulate electrode was determined to be best located 7.5 mm anterior to $A-P_E$ and 1.0 mm dextrad to S_o .

e. The parietal electrode was located 2.5 mm anterior to $A-P_E$ and 3.0 mm dextrad to S_o .

f. The occipital electrode was located 1.5 mm posterior to $A-P_E$ and 1.0 mm dextrad to S_o . Since the sagittal sinus was known to vary slightly in position from subject to subject, some care was needed in locating the occipital electrode which lies near the sagittal plane. Accordingly, all EEG electrode sites were located to a precision of ± 0.1 mm or better (the EBS electrode was held to a tolerance of ± 0.05 mm or better).

g. For proper recording of monopolar EEG signals, it was necessary to provide a stable reference (or indifferent) electrode. The need was satisfied by a chronic implant consisting of a stainless steel electrode uninsulated over the distal 5 mm of its length, placed along the nose astride the periosteum in the plane of the sagittal suture, and at least 10 mm anterior to the cingulate electrode. Observation of other reference electrode sites confirmed that the nasion site appeared to result in minimal signal artifacts due to neuro-muscular voltages generated by sniffing, and thus this site was used in all experimental subjects.

The locations of the trephine holes for all electrodes were marked by placing a high-speed electric drill in the stage of the Kopf apparatus and using this drill to "centerdrill," but not penetrate, the skull at the proper stereotaxic dimensions.

The EEG electrode mounting holes were trephined by hand, using a pin vice with a No. 56 drill bit extending about 1.5 mm past the chuck jaws. When the dura was clearly visible, the exposed skull surface was carefully cleared of bone fragments and blood, and the dural (cortical) electrodes were screwed into the skull.

Next, the location of the trephine hole for the EBS electrode was rechecked for accuracy, and the hole was drilled in the same manner as were the EEG electrode sites. The area was again cleaned of bone fragments and blood, and the dura was penetrated to a depth of 3 mm by use of a Yale No. 30G, 1/2-in. surgical needle.

The electrode cap with the cortical leads and the stimulus electrode attached was placed in the stage of the Kopf apparatus, and the stimulus electrode was cut to a length of 10 mm, measured from the connector base.

The stage was lowered⁷³ until the tip of the electrode was at the previously determined level of D_E and a final check of $A-P_E$ and S_E was made. The stage was then lowered until the EBS electrode tip penetrated the brain to a depth of 9.2 mm. This excessive penetration was done to assure that there would be no artifacts resulting from mechanical stress of neural tissue in the vicinity of the exposed electrode surface.⁷⁴

Finally, the stage was withdrawn until the electrode tip was positioned at 8.7 mm below mean skull height, and dental acrylic was placed under and around the electrode cap, with due care exercised to prevent the toxic acrylic from entering the EBS trephine hole.⁷⁵

⁷³ As the stage was lowered, the EEG electrodes were inserted into the appropriate connector cap pins and the leads were dressed to preclude short-circuit contacts as the cap descended to its final location.

⁷⁴ Necropsy of a few subjects revealed that gliosis soon filled the neural gap thus generated, and no loss of stimulus effectivity was evident.

⁷⁵ Bone wax was inserted in the EBS trephine hole to preclude such leakage.

When the dental acrylic had solidified, the surgeon again cleaned the exposed skull surface with alcohol. The scalp was then sutured, making sure that the acrylic adhesive was covered and that the electrode mount was clear.

Each animal was carefully observed for evidence of shock or dyspnea for 1h postoperatively and was then returned to its assigned living area. Each subject was examined daily for a period of 2 wk for signs of infection or erratic behavior. Nearly all subjects recovered without need of medical intervention (Figure 8).

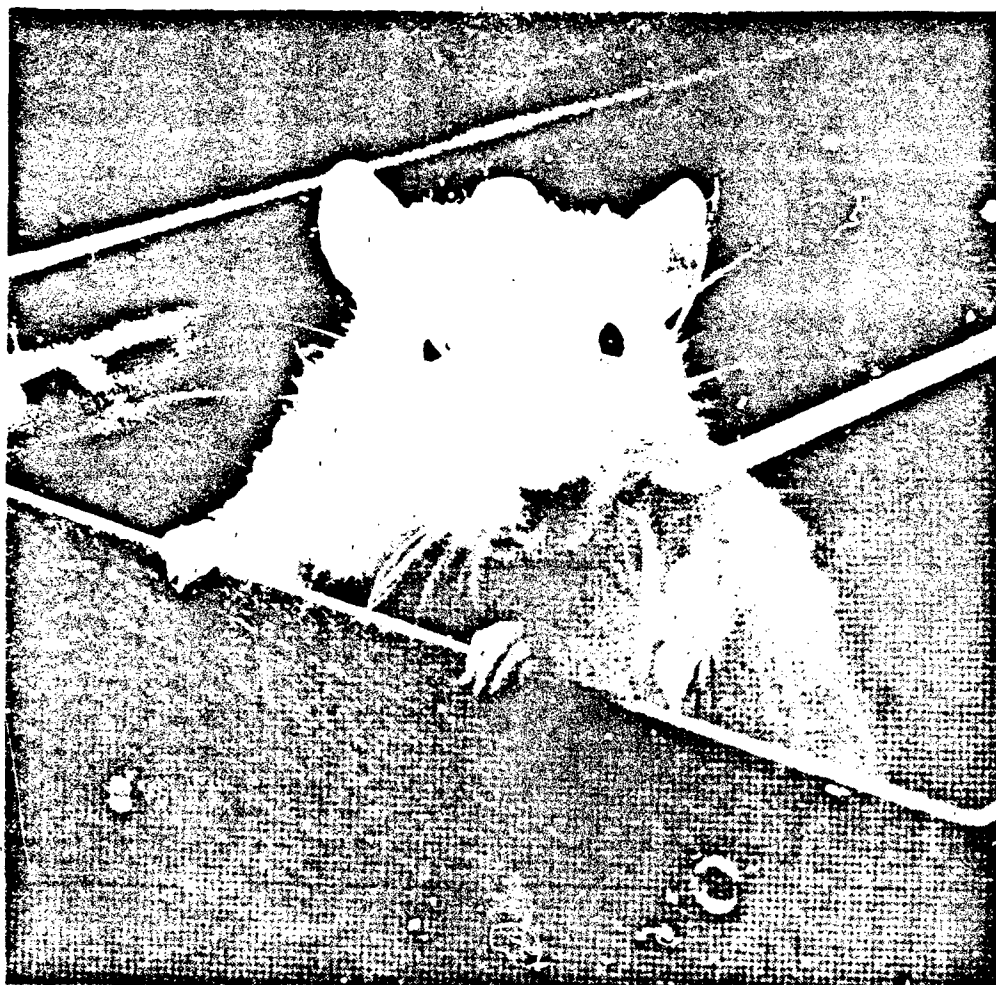


Figure 8. Post surgical subject.

16. EBS and Olfactory Stimulus Systems. Figure 9 schematically depicts the stimulus systems employed in this research. As shown, three olfactory stimuli were available for presentation to the test subject (located in the Faraday Box) on a random selection basis. The heavy lines in the figure denote lengths of "TYGON" tubing; the light lines represent electrical connections.

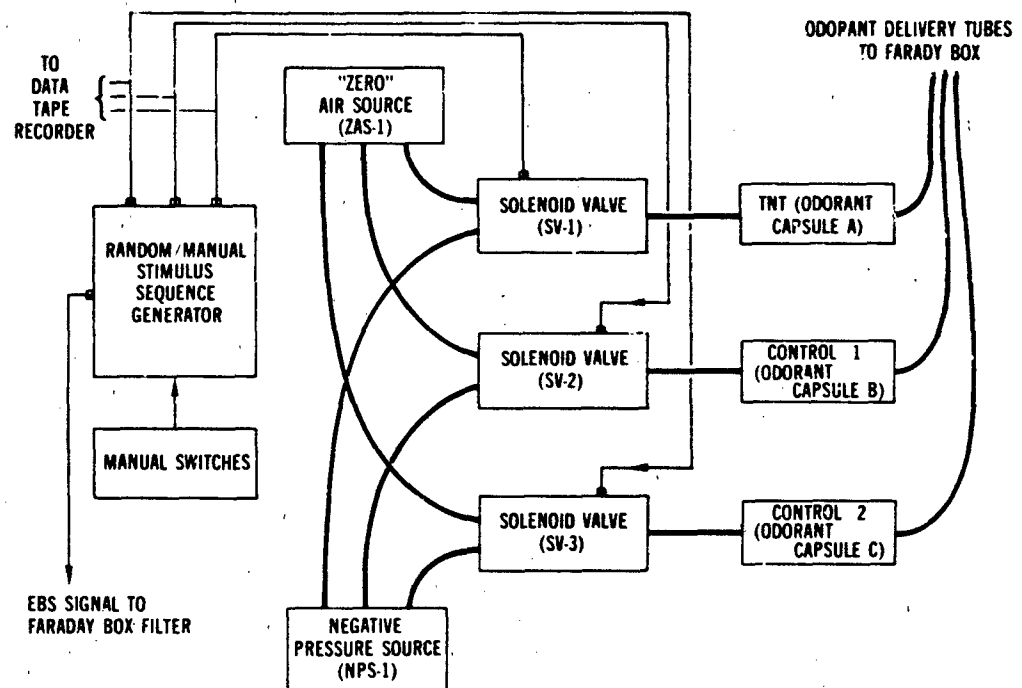


Figure 9. EBS and odorant delivery systems.

Both automatic and manual control of odorant and EBS stimuli were allowed by this instrumentation. During the shaping and conditioning protocols, the sequencing was accomplished automatically while manual control of odorant delivery was employed during data runs (EBS was not applied in these runs).

Each odorant delivery system consisted of a three-port rotary solenoid valve and an odorant capsule. The odorant capsules contained a few milligrams of the following odorants:

<u>Capsule</u>	<u>Odorant</u>
A	Flake TNT (military grade)
B	Coarse pine or cedar sawdust
C	Asphalt

Each of the odorant-control capsule/valve assemblies was supplied by two air-pressure systems. One air system delivered positive pressure "zero" air to the odorant capsule when the solenoid valve was energized to cause odorant delivery. The other air system presented a negative pressure to the odorant capsule when the solenoid valve was not energized, thereby assuring that no residual odorant-laden air remained in the delivery system during the inter-stimulus intervals.

Thus, the olfactory stimulus system generated a bolus of odorant-laden air which was delivered into the air ambient to the head of the test subject. A delay of 500 ± 70 ms existed between the time of solenoid energization and the time of arrival of the leading edge of the stimulus air bolus. This factor assumed great significance during signal analysis. (See Section III, "RESULTS.")

The entire interior of the Faraday Box was constantly maintained at a slight negative pressure and the exhaust air was released outside the laboratory in order to prevent, or at least minimize, a build-up of TNT throughout the adjacent test area. At the conclusion of each test, the entire apparatus (Faraday Box, restraint capsule, odorant stimulus generator, delivery system, et al.) was thoroughly cleaned to preclude odorant build-up and, hence, data distortion. No attempt was made to preclude laboratory ambient air from entering the test enclosure and "zero" air was used only in the odorant delivery system. The rationale for this action was simply that the animal was trained in the laboratory to detect TNT vapor in room air as it would be required to do in explosives detection service, and it would add nothing to the overall experimental results to use a sterile ambient environment.

While no attempt was made to exclude the routine odorants present in the laboratory, it should be noted that TNT was a unique substance in the building as far as could be determined. There was not even minute quantities of substances containing nitro (NO) groups (which could have possibly permeated the test area and caused masking or confusion in identification) present, nor had there been within the past 5 yr. It is virtually certain, therefore, that the test subjects were aware of the presence of TNT only from the odorant delivery system.

Military grade TNT is not a reagent grade substance and contains two major impurities: dinitrotoluene (DNT) and mononitrotoluene (MNT), each of which has a higher vapor pressure than does TNT. However, the quantities of these impurities present in all samples of military grade TNT, both foreign and domestic, show that the total amount present is less than 2 percent of the total mass so that these related compounds affect the total odor only slightly. This is a moot point, since DNT and MNT are always present in military grade TNT and, therefore, these compounds are part of the "normal" odor of TNT.

It was not the purpose of this experiment to test the subjects against a wide variety of odorants; therefore, they were not tested for their response to any odorants other than those listed above.

The EBS signal was generated in the Stimulus Sequence Generator and delivered to the subject by shielded leads to the connector panel on the Faraday Box. As noted previously, EBS was not applied during data runs, but the test setup is identical for both conditioning and data runs.

It is important to note that there was a finite but indeterminate possibility that some or all of the test subject population might eventually learn to cue on the sound of the solenoid relay which delivered TNT. In order to preclude the occurrence of such activity, the relays were switched in a random fashion from time to time so that no one valve delivered TNT vapor for every test run. It was, of course, necessary to thoroughly purge the relays prior to this functional change so that previous odorants which might cling to the valve assembly did not become test artifacts. The same pieces of TYGON tubing was used in all runs to deliver the odorants to the Faraday Box test enclosure.

17. Behavioral Shaping. When the test subject was completely recovered from implant surgery—a matter of 2 to 3 wk—the process of conditioning (following the rationale described earlier) was begun. This process consisted of two basic phases, the first of which used operant conditioning and the second of which used classical conditioning. Operant conditioning demonstrated the following important factors:

- a. The accuracy and adequacy of all electrode implants.
- b. The ability of the test subject to detect the desired target substance.
- c. The willingness of the subject to respond to odorant stimuli where brain electrostimulus was the single reward.

Classical conditioning was later applied only to those animals which successfully met the above criteria.

The need for classical conditioning originated from consideration of the ultimate use of the animal, which was, as noted previously, as a portable vapor detection system. The proposed use, schematically depicted in Figure 10, would result in considerable jostling of even a severely restrained animal as it was carried through a search pattern, and in this event, operant signalling could be seriously disrupted. Thus, it is highly desirable that the animal signal its awareness of target presence by a change in its cortical activity, and it was decided that classical conditioning would enhance this effect.

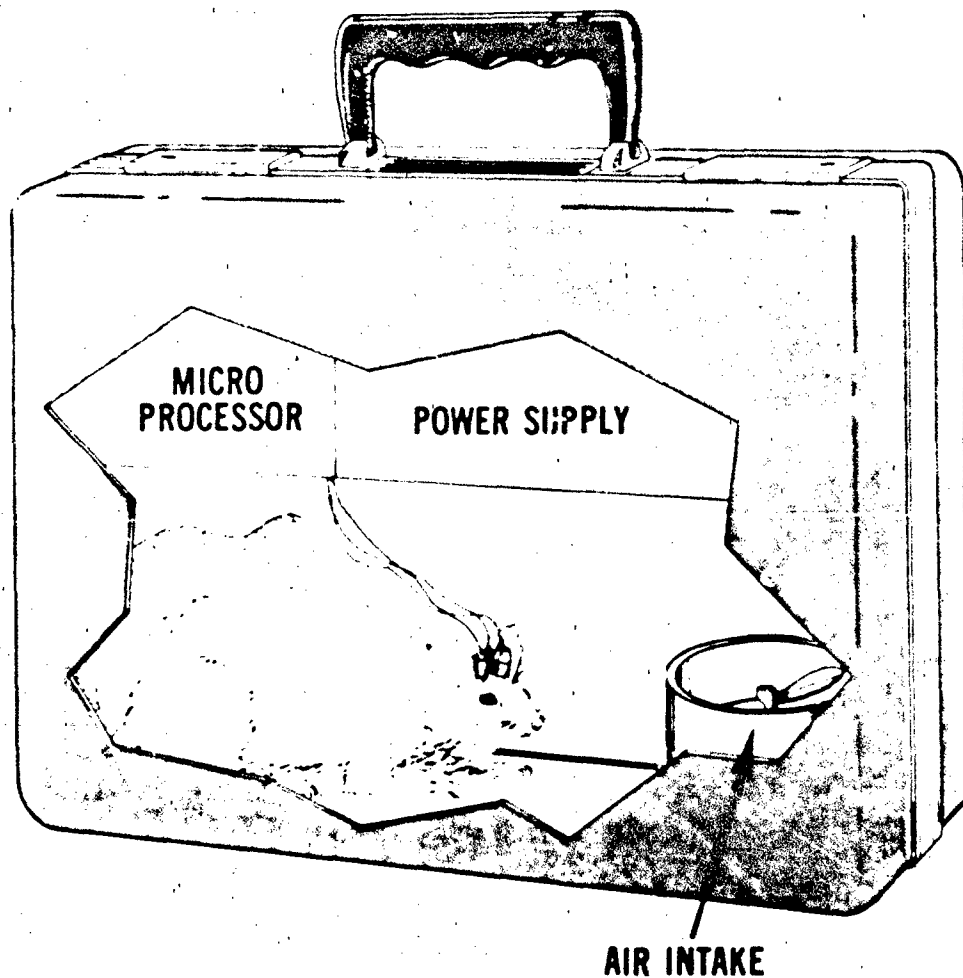


Figure 10. Proposed use of rat as explosives detection system.

When the post-operative subject was first connected to the stimulus generator, it was placed in a Skinner Box where a self-stimulus press bar was available (Figure 11). After a brief period, the animals generally accepted both this restrictive environment and the presence of the skull cap with the electrode leads. When the subject was fully adapted to the test box (as evidenced by the initiation of the self-grooming process with which this species occupies most of its "relaxed state" time), the first EBS was applied manually by the test operator. The effect of this stimulus was dramatic in every case of effective implant.⁷⁶ The animal often ceased all activities and remained motionless, usually in a standing posture, for several seconds. Cautious grooming then resumed. With the second application of EBS, the animal usually appeared perplexed but not alarmed and tended to scurry about the test cage sniffing rapidly. Subsequent stimulation caused evidence of increasing excitement.



Figure 11. Test subject undergoing behavioral shaping.

⁷⁶ Imprecise EBS electrode implant was readily apparent at this early stage of the conditioning protocol. If the subject manifested symptoms of fear, ataxia, seizure, or bizarre behavior, the implant was incorrect, as was also the case if the subject exhibited no behavioral change with EBS applied. Of the few who exhibited a fear response, some were able to derive the pleasure response with reduced stimulus current amplitude, while others always exhibited fear and were thus useless to the research. In all, over 70 Sprague-Dawley male albino rats were tested for implant effectiveness in this manner, and 12 were found to be of no experimental value. The reasons for these failures were not confirmed by histological examination, but were assumed on the basis of experience to be most probably due to:

1. Surgical error in electrode location.
2. Structural variation in the brain.
3. Brain trauma resulting from implant.

Assumptions 1 and 3 seem valid since the losses occurred largely in the first 30 subjects. Assumption 2 is valid since frozen section specimens obtained from other sources show some variation in the exact location of the MFB; the result of electrical stimulus of structures adjacent to the MFB is often a fear response.

Those animals which responded well to manually applied EBS soon began to exhibit the phenomenon of "superstitious behavior" which is remarkably similar to the behavior pattern of humans subjected to an unrecognized challenge. The animals obviously enjoyed the EBS, and they apparently assumed that the pleasurable event was solely the result of some action on their part. Immediately following an EBS, the subject would begin an attempt to reconstruct the events leading to EBS. Some rats ceased all activity for up to a minute, while others immediately began a series of unusual posturings at various locations in the Skinner Box.⁷⁷

In order to assist the rat in achieving self-stimulus in the minimum period, the test operator first applied 20 to 30 EBS regardless of the activity, posture, or position of the animal. After this, EBS was applied a like number of times only when the rat was oriented with its head toward the press bar. Next, EBS was applied only when the animal was within reach of the bar, and then it was applied when the rat investigated but did not activate the bar. Eventually, with this assistance from the test operator, the animal depressed the self-stimulus bar, and, after three or four such events, all subjects recognized the press bar as the source of stimulus.

This process was allowed to proceed in the case of most test subjects both for reasons of convenience—a simple, available Skinner Box could be used—and for purposes of observation. In the final stages of the second experimental phase of this research, the subject was confined in a small cage with its head oriented toward the self-stimulus treadle. The automatic stimulus sequencer was allowed to stimulate the animal at random intervals without human intervention. The subjects so shaped required about the same time for establishing the pleasure/treadle relationship as did those subjects shaped in the larger Skinner Box. The significance of the success of this procedure using the smaller cage is that a fully automatic training protocol could be employed to train detector rats en masse for use in field-deployable detection systems, thus verifying Thesis C.

There can be no doubt to any observer of the monumental pleasure derived by the test animal from EBS. Within a few hours (< 4) of their first EBS experience, the test subjects were self-stimulating at very high rates. Indeed, 1300 to 2000 bar presses per hour were not uncommon for the average subject—and these animals would, if allowed to do so, continue this self-indulgent behavior until they collapsed from fatigue or dehydration. Such behavior argues that EBS must surely be the "ultimate" conditioning stimulus.

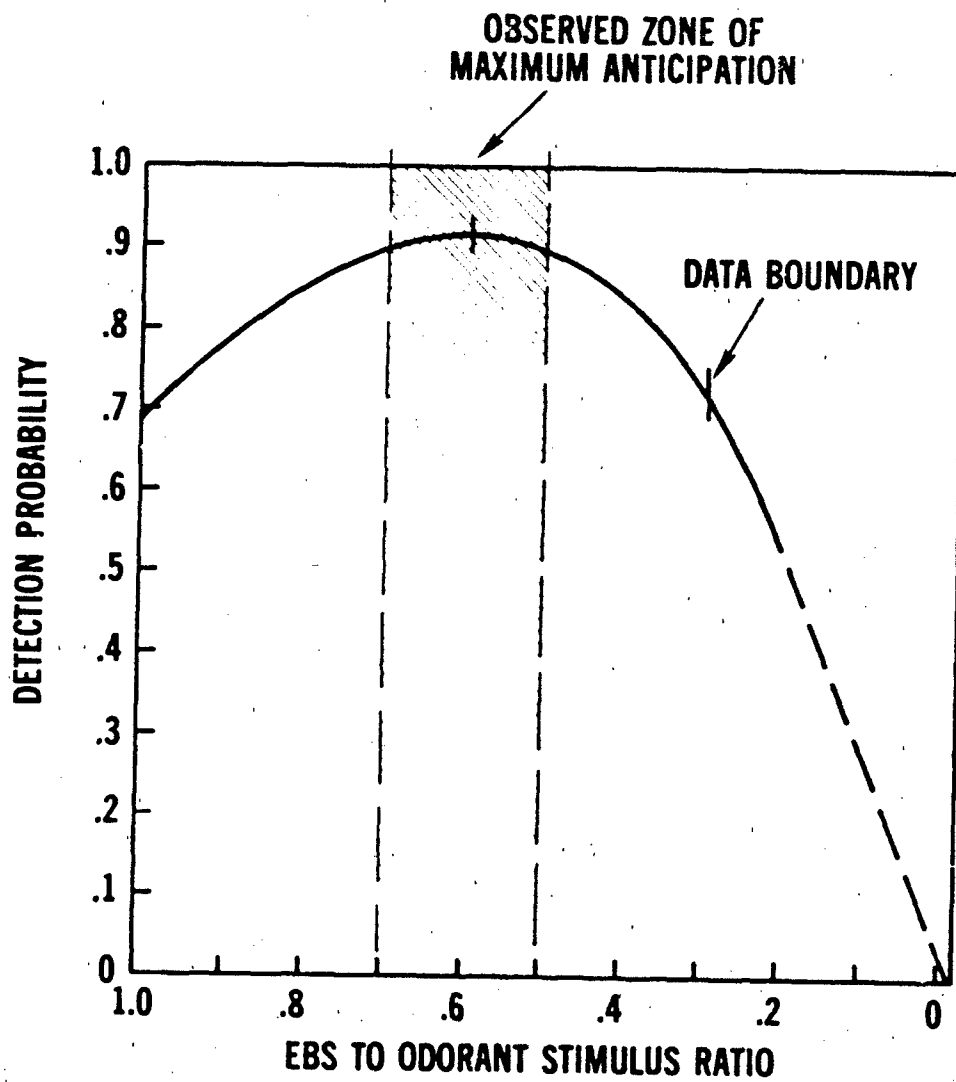
⁷⁷ It is not difficult to image that a very similar form of classical conditioning (sans EBS, of course) is the mechanism of origin in human superstition and of the sometimes bizarre behavior which results from unexplained, unfamiliar occurrences.

18. Operant Conditioning. After the subject was fully aware of the pleasure to be derived from the bar-press activity, the shaping period was concluded and operant conditioning began by simply allowing no EBS in response to a bar press unless TNT vapor was present in the odorant delivery port of the Skinner Box. As expected, when an EBS did not result from a press, episodes of somewhat frantic bar pressing was interspersed with periods of quiet contemplation or feverish resumption of the earlier superstitious search for the source of pleasure.

In order to verify that only the presence of TNT vapor in the test fixture would induce the desired behavior, other odorants were delivered in the exact manner as was TNT vapor. Pine and cedar sawdust, asphalt, and even food were used as neutral olfactory stimulus because the animals were known to sniff these substances in their normal life patterns. After about 1 h of multiple odorant exposure, the animals completely ignored any odorant which was not associated with EBS availability; however, there were occasional false alarms which probably resulted from a decision by the subject to determine if EBS might be available in spite of the presence of a "wrong" odorant.

In the surprisingly short space of about 40 h, most successfully conditioned rats were able to recognize the cause-and-effect relationship between the presence of TNT vapor and the availability of EBS in response to bar press. Additional operant conditioning (10 h) was given to each subject (30 min per day for 20 days); during this time, the animals were gradually transitioned from 100 percent EBS (an EBS each time TNT vapor was present) to an EBS-stimulus ratio of 0.6 (see Figure 12). This reduction of reward frequency was in accordance with the well established precepts of both operant and classical conditioning, which state that excessive reward can result in a rather substantial diminishment in performance due to habituation with a resultant diminishment of anticipation. It appeared, after some experience with this strain of rats, that a reward in about 80 cases out of 100 was the limit beyond which some habituation would surely occur. Since a high degree of anticipation of reward was critical to the research, a 0.6 reward/odorant stimulus ratio was selected and maintained throughout the experiment.

19. Classical Conditioning. In this final phase of conditioning, those test subjects which accepted operant conditioning were placed in the restraint capsule (Figure 4) where no provisions for self-stimulus existed. As before, odorants were randomly introduced into the test fixture, but in these runs, EBS was delivered gratis during 60 percent of the deliveries of TNT vapor. The animals were apparently not vastly confused by the non-operant presence of EBS and soon adapted to the new test conditions. After about 4 h of classical conditioning, most of the test subjects were deemed ready for data-taking sessions.



(ALL SUBJECTS BEGAN TEST WITH 40 HOURS
OF CONDITIONING AT UNITY RATIO.)

Figure 12. EBS/olfactory stimulus relationship.

All told, a total of 60 to 80 h of conditioning (operant plus classical) was found to be adequate to transform naive subjects into skilled and discriminating sensors of TNT vapor. Not all subjects which could be shaped were usable, but the total yield useful for testing from those animals which could be shaped was better than 80 percent—a total far in excess of that originally anticipated. Overall, the percentage of animals usable as sensors relative to the total naive population was about 50 percent. As a comparison, the same ratio for dogs trained by non-EBS methods was about 15 percent and the training protocol required in excess of 8 months.⁷⁸

Extinguishment of conditioning was not a subject of investigation during this research except peripherally in determination of the optimum reward/olfactory stimulus ratio. It is significant that no significant extinguishment of conditioning was experienced in any subject, even in the unique case of an early subject named "Speedy." Speedy was retired from service during a lull in the research, and he became a pet of the custodial assistant. After 1 yr of total inactivity as a test subject, Speedy was again placed in the operant experimental chamber and subjected to TNT vapor. He immediately recognized the odorant, pounced upon the stimulus press bar, and attempted to stimulate at a rate approaching 2000 presses per hour. This astonishing performance continued for several weeks, but before he could be placed on an EEG schedule, he died from the effects of a respiratory viral infection which claimed three other skilled subjects. Various other subjects were carefully evaluated after inactivity periods of up to 6 wk, and none exhibited any evidence of severe extinguishment. From the performance of these subjects, one must conclude that the effects of EBS in conditioning are potent indeed, and that the data obtained in this research are free from the effects of extinguishment of either operant or classical conditioning.

20. Data Recording System. It is difficult to obtain quality recordings of animal cortical activity in spite of the fact that the signal levels are higher and of less diffuse origin than those obtained from the conventional scalp electrodes used in human clinical practice. The major problem in each instance is generally one of signal artifacts which can mimic or mask the desired signals.

Much of the problem of signal artifacts in the case of animal EEGs arises as a result of the motion of even a severely restrained test subject, which results in the presence of signals of both cortical and muscular origin. Additionally, there may be triboelectric signals generated by motion translated along the EEG input signal leads. Although this effect is usually of secondary significance, these microvolt signals can add significantly to the total artifact content of the data recordings. As explained earlier, motion artifacts were minimized by use of the device shown in Figure 4.

⁷⁸ Nolan, R. V. and Gravitte, D. L., "Mine Detecting Canines," Report 2217, U.S. Army, MERRICOM, Fort Belvoir, Virginia (1977).

Once the problem of motion artifacts was reduced to a minimum, it was a simple matter to devise and fabricate a screened enclosure (see Figure 13)—a Faraday Box—which would achieve at least a 70-db to 80-db attenuation of artifacts derived from sources in and adjacent to the laboratory area. The Faraday Box was built with a wooden frame covered inside and out with electrolytic copper (99.5 percent or better chemical purity) except for a viewing port in the lid, which was covered with bronze wire screen both inside and out. All metallic junctions on both the main body and the lid were carefully joined and soldered to assure complete electrical continuity. The efficacy of the shielding was found to be such that no artifacts were evident in the EEG signal preamplifier output in any channel when high-intensity noise sources were placed proximal to the closed test box and with preamplifier gain set at 10,000.

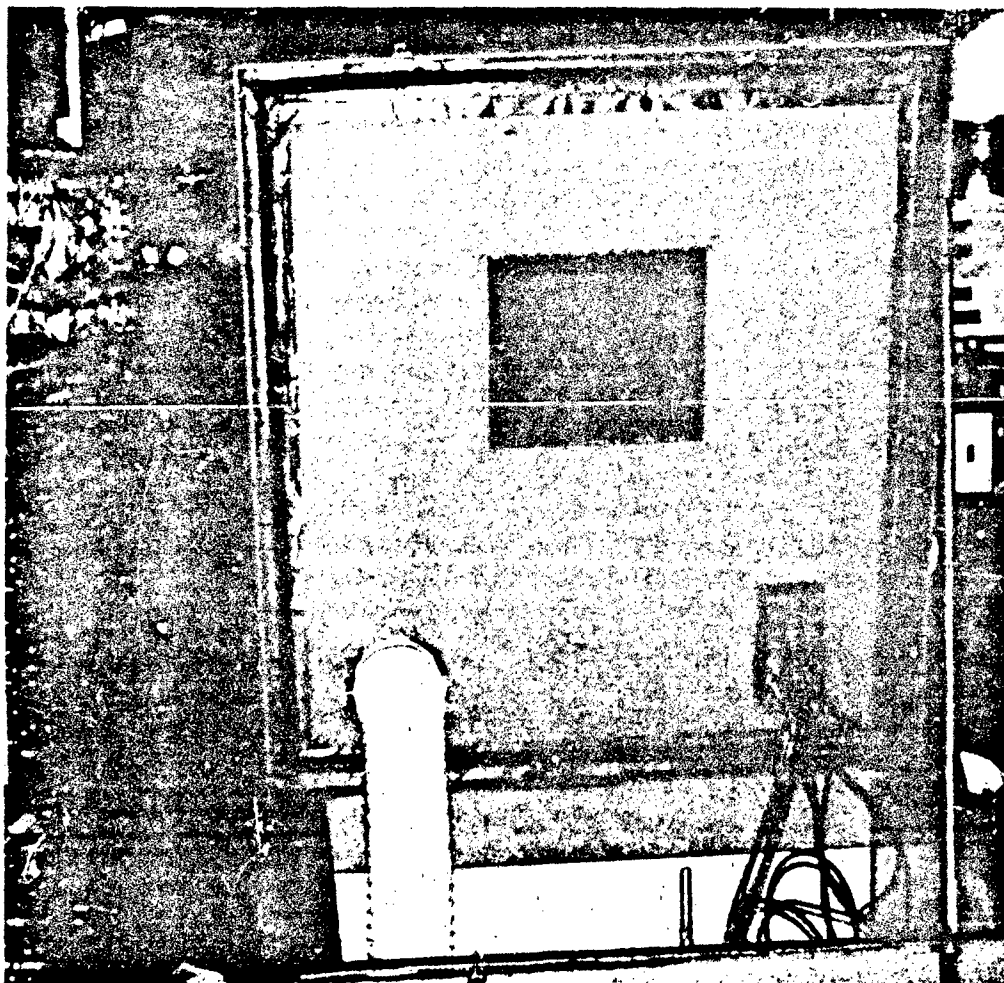


Figure 13. Faraday box test enclosure.

In order to assure the total absence of power line noise, a high-quality, battery-powered, variable-bandwidth preamplifier was selected which could be placed within the Faraday Box during test runs. The amplifier chosen for this service was the Princeton Applied Research (PAR) Model 113 Low-Noise Preamplifier. This unit is advertised to have a common-mode rejection of 120 dB for gain settings in excess of 200, a gain accuracy of ± 2 percent and a total distortion of 0.01 percent. The amplifier can operate normally for at least 8 h on its internal battery, and this battery can be fully recharged in 16 h.

It should be pointed out that even though a commercial 10-channel EEG apparatus (Grass Model 7) was available for these tests and was, in fact, used early in Experiment I, it was determined by later experiment that the lowest possible system noise level was obtained by use of the PAR amplifier located within the shielded enclosure.

The cortical EEG signals, as read from the dura meter of the test subjects, were in the order of 20 μ V to 100 μ V, and since the PAR 113 was routinely operated at a gain of 10,000, the resultant large output signals (200 mV to 1000 mV across 600 ohms) virtually precluded the introduction of laboratory artifacts into the input of the data tape recorder. Great care was taken to eliminate ground loops and other "hidden sources" of noise input to the data system.

When magnetic tape data recording systems were first considered, the use of direct digital recording was contemplated since this data form would simplify data analysis by precluding the need for post-experimental digitization, and it would also allow for some "real time" data analysis in the physiology laboratory. After due consideration, it was determined that these were only slight advantages among many severe disadvantages inherent in direct digital recording.

A major consideration was the cost and the availability of digital recording equipment and the cost, difficulty and hazards of transporting such systems from one facility to another. Additionally, if one wished to acquire all available data, the amount of digital tape used would be staggering, compared to the amount of tape required for analog recording.

At a data rate compatible with the anticipated frequency response of Experiment II (300 Hz) each digital tape would have run 4 min or less, using the equipment available; in contrast, comparable analog tapes would run about 6 h each.⁷⁹

⁷⁹ Since the spectral density of the EEG signals derived in each experiment was unknown prior to data analysis, the digital sampling rate was at first indeterminate. If, indeed, the EEG contained frequencies up to 300 Hz, then the optimum sampling rate had to be at least 750 samples per second, which required a true sampling rate of 1024 samples per second. On the other hand, if the usable frequency spectrum was truncated at or below 200 Hz, it would be possible to sample at a 512-Hz rate, thus halving the amount of digital recording tape used.

Since there was no a priori reason to believe that usable frequency components did not exist beyond 200 Hz, a sampling rate of 1024 per second would have been indicated, thus resulting in the 4-min tape life noted.

Consideration of available EEG data led to the conclusion that the use of IRIG (Inter Range Instrumentation Group) low-band analog recording would be ideal for this research, since there was no basis for postulating the presence of significant frequency density past 300 Hz, and IRIG low-band recording allows a system bandwidth ranging from d.c. to 312 Hz (6-db/octave high-end roll-off). Additionally, this type of recording allowed for the use of analog direct recording for ancillary data, such as the oral commentary of the test operator and a simple time reference code. From the foregoing considerations, analog recording was selected for the basic data collection system.

Consequently, the data recorder used was a Honeywell Model 7600, 14-channel, FM, magnetic analog tape recorder operated in the IRIG low-band mode, which had the following operating parameters: tape speed 1-7/8 in./s; center frequency 1.68 kHz; data bandwidth ± 1 db from 0 Hz to 312 Hz; S/N 45 db. In this mode, the manufacturer claims 1.2 percent total harmonic distortion, ± 0.5 -percent deviation linearity, and ± 0.5 -percent d.c. drift over 8 h. These specifications are entirely adequate for the purpose of this experiment.

All data were recorded on pristine 3600-ft reels of Ampex Type 760, 1-in. precision magnetic tape.

Since recording and playback were to be accomplished on different machines in different laboratories, it was necessary to record calibration signals on each channel so that playback output signals could be held to the closest possible facsimile of the input signals. Consequently, immediately prior to each data run, all FM signal channels were calibrated by first applying a direct short circuit to the recorder input jacks. The individual channels were then adjusted to give zero amplitude output. Following this 5-min segment was a 5-min segment wherein a 200-Hz, 50- μ V signal was applied to all three PAR amplifier inputs simultaneously and the data recorder channel gains were adjusted for identical recorder output amplitudes. The passband of the PAR amplifiers was extended in this recording segment so that the high-frequency roll-off was 1000 Hz while gain remained at 10,000 X. At the conclusion of the calibration sequence, the passband of the preamplifiers was set to give 0.3-Hz low-frequency roll-off and 300-Hz high-frequency roll-off (12 db/octave).

In order to facilitate the process of locating any given data epoch on the large tape reels, it was necessary to include a precision time code on each data tape. The IRIG-B time code format was selected on the basis of available equipment (Systron-Donner Model HI-150 time Code Generator). The timing signal was directly recorded on head track 10 of all data tapes.

To further enhance the overall data set utility during playback, a continuous commentary was recorded by the test operator during every data run. No comment was regarded as too trivial to record during these sessions and many motion artifacts were readily identifiable in the reproduced data by virtue of the oral annunciation of test subject activity.⁸⁰ Voice data were recorded on all data tapes.

It was necessary to identify precisely the time of activation of the odorant control valves so that data epochs could be selected which covered only a few seconds prior to and after odorant delivery. Consequently, the odorant release solenoid valve d.c. control voltage was simultaneously applied to the solenoid coil and to an FM data track. These d.c. signals, used in concert with the IRIG-B time code, allowed for precise positioning of the data tapes during playback with the result that the total data digitization effort was reduced to a simple and efficient routine.

A schematic representation of the entire recording system is shown in Figure 14 where the system is connected for monopolar signal recording in Experiment II.

21. Data Recording Procedures. The total EEG data set resulting from each of these experiments was contained on analog data tapes. Additionally, written data sheets of the type shown in Figure 15 were prepared to aid in data epoch location and to record incidental information not found on the data tape voice channel. Data were acquired on a total of 10 test subjects in each experiment, and from the mass of data thus acquired in Experiments I and II, over 1300 usable data epochs were identified as appropriate for digitization and data analysis. Data analysis involved about 500 epochs from the first experiments and 800 epochs from the second experiment.

The EEG signal data were recorded either in monopolar or in bipolar format on FM channels 7, 9, and 11 of the Honeywell 7600 data tape recorder. The recording speed was 1-7/8 in./s, which permitted recording a total of nearly 6 h of data per data tape.

Monopolar recordings were made using the nasion lead (see Figure 2) as the indifferent electrode. During bipolar recordings, the nasion lead was connected to the system ground. The EBS electrode was not connected to any lead during any data run. The resulting analog data set then consisted of the following EEG recordings:

⁸⁰ The test operators were asked to investigate the seemingly ludicrous possibility that eventually the subjects might learn to recognize the words uttered by the various test operators as they announced the nature of the impending stimulus, and at various stages of testing. On occasion, various operators approached the Faraday Box and stridently announced (falsely) that the next test would be a TNT event. Also, subjects placed in a Skinner Box were accorded the same treatment. At no time was there any behavioral evidence (such as violent sniffing or lunging at the Skinner Box treadle) that the subjects could cue on oral stimuli. Perhaps it was well that this test was performed, since the question of oral stimulus has arisen in various discussions relative to this research.

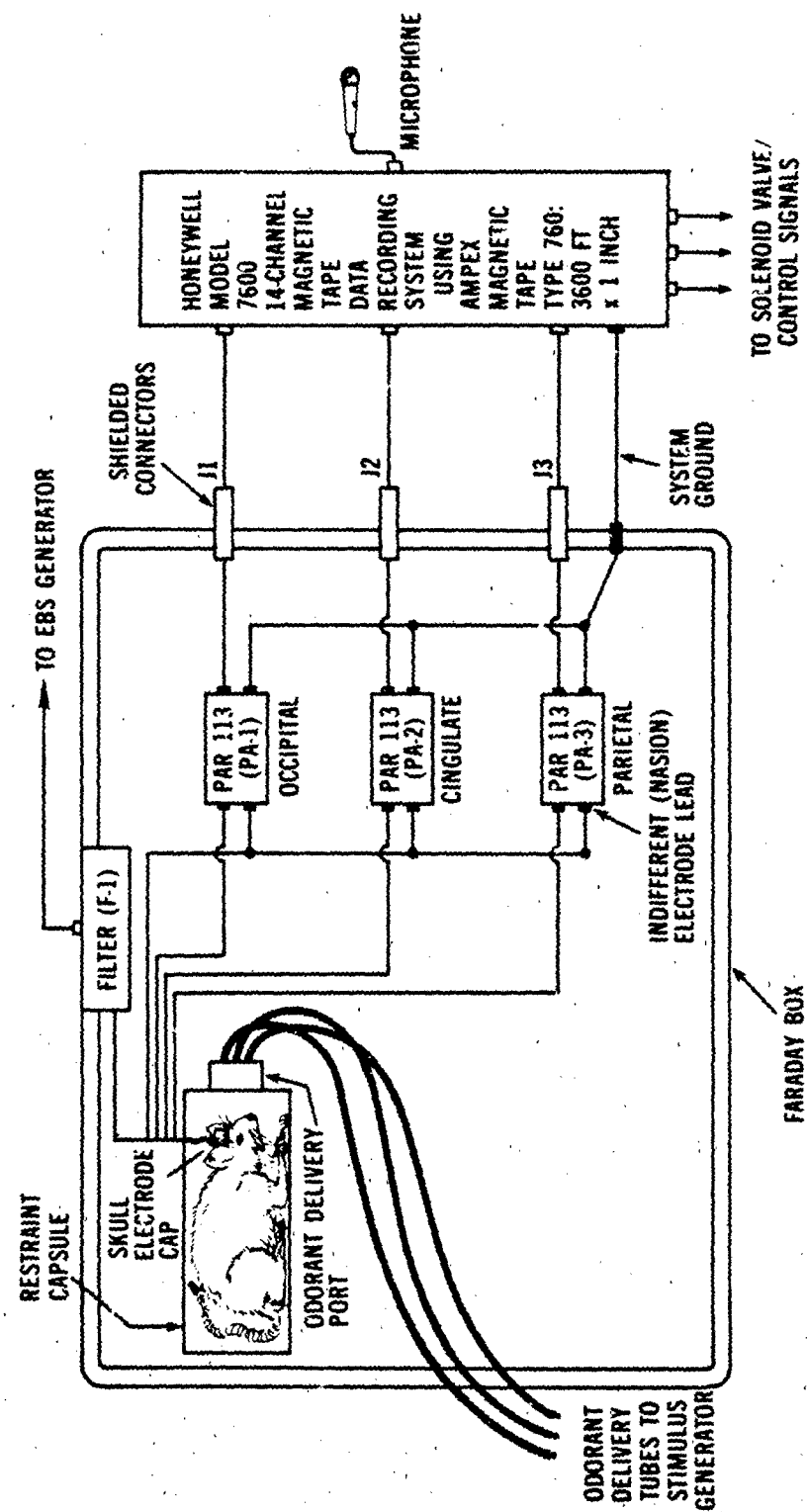


Figure 14. Data recording system.

AESC RESEARCH PROJECT
R. V. NOLAN

TAPE NO: 5A DATE: 6 APR 79 PAGE NO: 1
 RAT NO: 9 SESSION NO: 1 RECORDING TYPE: MONO
 TEMP: 70°F HUMIDITY: 80% REL
 EXPERIMENTERS: R. NOLAN, K. WELSTEIN

PREAMP STATUS

UNIT NO	GAIN	ROLL OFF	
		LF	HF
34113	10K	0.03 Hz	300.0 Hz
34114	10K	0.03 Hz	300.0 Hz
34115	10K	0.03 Hz	300.0 Hz

RECORDER STATUS

UNIT	TAPE SPEED	BAND WIDTH	CENTER FREQUENCY
HY 7600 # 16764005	1 7/8 IPS	H1: 312 Hz L0: D.C.	6.75 KHz

HEAD	MODE	DATA	HEAD	MODE	DATA
1	FM	CAL (20042)	8	-	-
2	-	-	9	FM	EEG PARIAL
3	-	-	10	AM	TIME CODE
4	FM	TNT MARK	11	FM	EEG SINGULATE
5	-	-	12	-	-
6	FM	NGWT MARK	13	-	-
7	FM	EEG OCCIP	14	AM	VOICE

DATA SEQUENCE

PRINCIPAL EVENT	START		STOP	
	TIME CODE	FOOT COUNT	TIME CODE	FOOT COUNT
DC CALIB	00:00:00	144	00:05:13	195
AC CALIB	00:06:43	210	00:11:19	252
EEG BASELINE	00:23:41	366	00:28:41	415
EEG PRE TNT	00:29:12	415	00:49:00	681
TEST SEQUENCE BEGUN AT				
	00:31:00			

Figure 15a. Sample recording data sheet.

STIMULUS SEQUENCE

PAGE NO: 2

TAPE NO	EAT NO/ SESSION NO	TIME CODE		ORDER CODE	TRAINING PARAMETERS
		TNT	CONTROL		
5A	G/1	00:31:00	—	T	[NO TRAINING
		—	00:31:42	N	IN EOS/TNT
		—	00:32:30	N	PAIR PRIOR TO
		00:33:15	—	T	THIS SESSION
		—	00:33:59	N	(1)
		00:34:45	—	T	50 TNT & 30
		00:35:20	—	T	NEUT PAIRINGS
		—	00:35:55	N	PRIOR TO (1)
		00:37:08	—	T	(100% R)
		—	00:37:50	N	—
		00:38:35	—	T	(2)
		—	00:39:20	N	54 TNT &
		—	00:40:06	N	32 NEUT
		—	00:40:36	N	PAIRINGS
		—	00:40:36	N	PRIOR TO (2)
		00:40:58	—	T	(100% R)
		00:41:15	—	T	(3)
		—	00:41:29	N	20 TNT &
		00:41:59	—	T	12 NEUT
		00:42:20	—	T	PAIRINGS
		00:42:35	—	T	PRIOR TO (3)
		—	00:43:05	N	(100% R)
		—	00:43:30	N	—
		—	00:44:01	N	(4)
		00:45:00	—	T	27 TNT &
		00:45:40	—	T	20 NEUT
		00:46:05	—	T	PAIRINGS
					PRIOR TO (4)
					(100% R)
					—

Figure 15b. Sample recording data sheet.

STIMULUS SEQUENCE

PAGE NO: 3

TAPE NO	RAT NO/ SESSION NO	TIME CODE TNT	CONTROL	ORDER CODE	TRAINING PARAMETERS
5A	6/1	-	00:46:39	N	-
		-	00:47:10	N	-
		00:47:49	-	T	-
		00:50:00	-	T	(5)
		00:50:40	-	T	50 TNT &
		-	00:51:10	N	30 NEUT
		-	00:52:01	N	PAIRINGS PRIOR TO (5)
		00:52:40	-	T	(100% R)
		-	00:53:08	N	-
		-	00:54:40	N	-
		-	00:55:00	N	(6)
		00:55:20	-	T	54 TNT &
		00:55:40	-	T	32 NEUT
		-	00:56:20	N	PAIRINGS PRIOR TO (6)
		00:56:40	-	T	(100% R)
		00:57:53	-	T	-
		00:58:34	-	T	-
		-	00:59:02	N	(7)
		-	00:59:40	N	20 TNT &
		01:00:10	-	T	12 NEUT
		-	01:00:44	N	PAIRINGS PRIOR TO (7)
TEST TERMINATED					

Figure 15c. Sample recording data sheet.

Monopolar:	Nasion to Parietal
	Nasion to Occipital
	Nasion to Cingulate
Bipolar:	Parietal to Occipital
	Parietal to Cingulate
	Cingulate to Occipital

To allow for precise identification of stimulus odorant delivery initiation during data analysis, the odorant delivery solenoid d.c. control signals were recorded on FM channels 4 and 6. The solenoid signal from the TNT odorant system was uniquely recorded on channel 4, while the two control odorant initiation signals were "and-gated" and recorded on channel 6.

Voice commentary was recorded (direct AM recording) on channel 14, and the time code signal was always on channel 10.

A short sample of the recorded analog data is shown on Figure 16. This is a monopolar recording, and at the approximate mid-point of the sample time axis, the presence of TNT solenoid signal is evident as a step change in the channel 4 playback trace. (Figure 16 was not generated by a high-fidelity playback system. The pen recorder used had a bandwidth of only 100 Hz for small amplitude signals, and this bandwidth deteriorated with increasing signal amplitude.)

The voice channel contained much valuable information since the test protocol demanded that the test operators keep up a running commentary during each formal data run. At least 20% data epochs were eliminated from consideration during final data analysis as a result of warnings, observations, and comments on the voice channel.

The standard IRIG-B time code recorded on channel 10 was used to precisely time all recorded events, including recording system calibration and subject introduction. By this means, any event could be located on any analog tape with an accuracy of 1 s (2 in. in 3600 ft).

The term "data epoch" has been used repeatedly thus far in this text, and it is now pertinent to define this term: An *analog data epoch* is the total data contained in the time period extending from 10 s prior to the presence of a solenoid control signal to 10 s past the time of the control signal. Thus, Figure 16 does not depict an entire data analog epoch since the image size allows observation of about 9 s subsequent to the control signal and only about 8 s prior to the control signal.



CINCINNATI LEAD



OCCUPATION LEAD



PARIETAL LEAD

TIME = 08:46:17

TIME = 08:46:17

TNT SIGNAL

SUBJECT	CHART SPEED:
RATC HYDROPHOR RECORDING	= 2.5 in/sec
	CHART CALIB: 1 in = 10mV
DATA TAPE:	575104 BW:
3A	= 312 N 0 5 44

DATE = 2 MAY 1977

NEUTRON SIGNAL

Figure 16. Analog data sample.

The presence of the time code allows the simple extraction of these epochs from the great mass of background EEG data, and then it serves again as a backup identifier of the digital event.

Digital data epochs are defined in Section III, "RESULTS."

A typical recording session began early in the day with the pseudo-random selection of a prime and an alternate test subject. Over the complete course of the experiment, each animal spent a minimum of 4 periods in the test chamber, and thereby each subject contributed a total of about 4 h to the data bank.

The subjects were maintained on a diurnal cycle such that they were in darkness from 1800 h to 0600 h 7 days a week. This rigid schedule was adopted so that no confusion or anxiety would result from the sudden introduction of light just prior to a test sequence. Upon removal from the domiciliary area, the primary subject was placed first in the restraint device and then in the Faraday Box. Following this transition, each subject was given ample time to accommodate to the environment of the test chamber while preliminary recording setup and calibration were in progress. The data runs began only when the subjects were fully adapted to this milieu.

Several subjects tested in the formal data recording sessions were first presented to the test station as an individual totally naive to any form of olfactory stimulus, although each subject was fully conditioned to the effects of self-induced EBS, and each was adjudged to be properly implanted. (Additionally, it was determined during EBS training that each EEG electrode was satisfactorily implanted.)

The purpose of introducing the naive subjects directly to the test station was to obtain EEG background both prior to and during the first exposures to the odorants used in the experiments, and thereby to attempt to discern if there were any unconditioned or natural AESC in response to these odorants.

Figure 15 is a reproduction of a data set randomly chosen from Data Tape 5A (second experiment). Note that d.c. calibration occurred between relative time zero and 05 min, 13 s (00:15:13) and a.c. calibration then occurred between 06 min, 48 s (00:06:48) and 11 min, 19 s (00:11:19). This final system calibration was followed by 5 min of baseline EEG data, during which time the subject was allowed to adjust to the test chamber.⁸¹

⁸¹ In practice, this period was often as long as 1 h for those subjects which demonstrated an unusual reluctance to accept the restraint device. In these few cases, the calibration sequence was re-run, and the data sheet represents only the formal run as shown.

At the relative time 00:31:00, a "Pre-training" or "Naive Data Set" was initiated. The exact sequence of 15 TNT vapor and 14 control odorant exposures is detailed in Figure 15.

Unfortunately, but not unexpectedly, Rat G and most of his test peers in the naive state were very disturbed by the restraint—in spite of their thigmotactic nature—and the number and magnitude of the muscle artifacts resulting from their struggles to escape rendered much of the naive EEG data useless. An occasional epoch of value resulted from the fortuitous coincidence of cessation of struggle and odorant delivery. From these limited data, it was not possible to elucidate any statistically valid evidence of the AESC prior to training. It is important to note that these data were recorded for reasons of experimental completeness and were not considered as part of the formal data set, since there was no reason a priori to believe that the TNT target odorant would evoke an AESC in a naive subject.

Figure 15 shows the next series of tests on Rat G where the subject was no longer totally naive. In the column at the extreme right of the figure, the training parameters for this subject are presented. The symbols are to be interpreted as follows:

Prior to sub-session 5, which began at relative time 00:50:00, Rat G had received 50 exposures to TNT vapor and 30 exposures to the control odorant. Coincident with each TNT exposure, the subject was given EBS. Conversely, no EBS was given during the 30 neutral (control) odorant deliveries. The notation "100%R" indicates that EBS was given in every instance of TNT odorant delivery. As the animal became more experienced, this percentage was dropped until in the final sessions some days later, an EBS to TNT odorant delivery ratio of about 60 percent was established. This was done to prevent habituation and to accent anticipation, as noted earlier.

When sub-session 5 ended at 00:54:40, Rat G was then given 54 additional TNT vapor/EBS pairings interspersed with 32 control odorant exposures. Following this training, sub-session 6 began at relative time 00:55:00.

In this manner, the first day of Rat G's training and data acquisition cycle progressed to its termination at 01 h. 00 min. 44 s.

It is essential that the reader understand that the relative time displayed on the data sheets was for the convenience such markings present to data analysis, and that the time readings bear no resemblance to true chronological events *except* during data runs. Thus, it could have occurred that as little as 30 min. or as many as 90 min. elapsed in real time between the events defined as 00:54:40 and 00:55:00 in Figure 15. However, the 03 min and 24 s which are shown to have elapsed between 00:55:00 and 00:58:24 represent real time in that the events occurring in this data interval were an uninterrupted continuum.

Data acquisition proceeded in the manner described above until at least 100 stimulus events occurred in the case of each test subject. In addition, over 60 h of background EEG data were recorded as ancillary information in the event that extensive examination of diurnal (or basal) EEG variation became necessary.⁸²

22. Data Playback Systems. The data playback system for Experiment I is diagrammed in Figure 17. All data were recorded on a Honeywell Model 7600 Magnetic Tape Recorder, as described earlier.

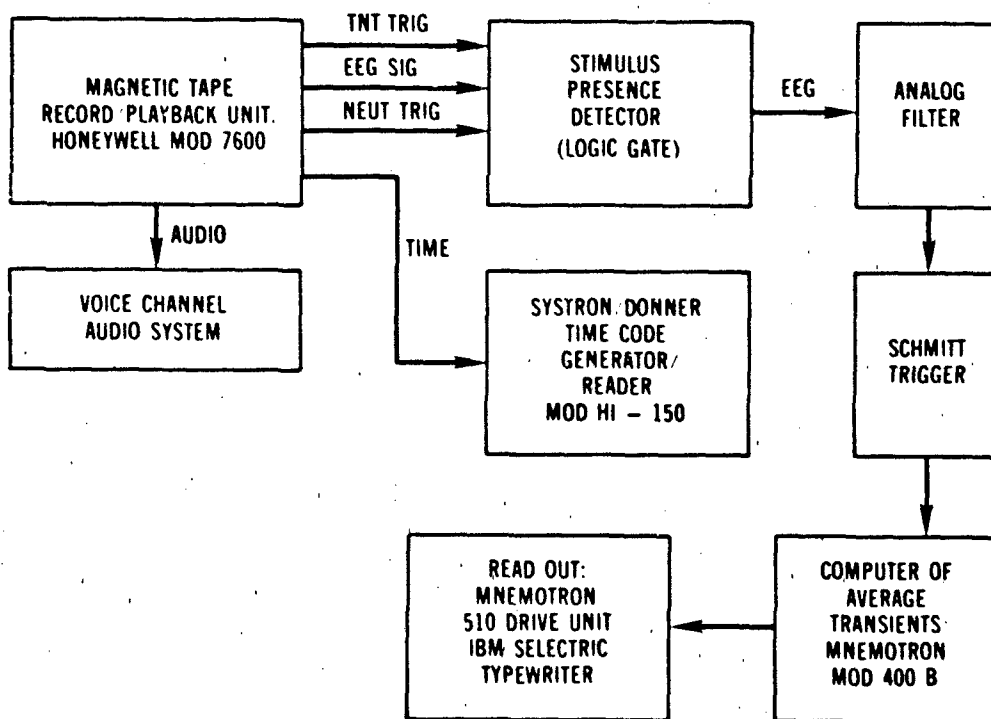


Figure 17. Data digitization and signal processing: Experiment I

The solenoid odorant control valve energizing signal was recorded on recorder channel 4 (TNT) or channel 6 (NEUTRAL), and this signal served as a precise trigger for the logic gate in the stimulus presence detector. The logic gate could be set to admit for analysis EEG data which consisted of pre-stimulus or post-stimulus segments. The analog

⁸² These latter data events were rather casually examined, since an obvious need did not arise for extensive background analysis as anticipated; however, the principle for "excess data where practical" was followed in this research, and the background data were simple and inexpensive to record.

filter was set to admit frequencies in 26 bands in the range 1.0 Hz to about 40 Hz to the Schmitt trigger. The Schmitt trigger squared those EEG signals with amplitudes above the average recorder noise background and thus provided a computer of average transients (CAT) with a trigger signal which was the analog of EEG frequency in the band of interest. The CAT began a sweep of addresses at a constant rate of 3.2 kHz upon receipt of a trigger pulse. The sweep continued until receipt of another trigger due to a zero crossing in the Schmitt trigger. In this manner, a histogram of timed intervals between baseline crossings was accumulated. The CAT combined proper addresses and delivered data for 26 bands of frequencies, which are shown in Table 3.

Table 3. EEG Frequency Bands - Experiment I

EEG Band	Upper Roll-Off (Hz)	Lower Roll-Off (Hz)	CAT Address
1	1.5	1.0	135-201
2	2.0	1.5	101-134
3	2.4	2.0	81-100
4	3.1	2.4	67-80
5	3.6	3.1	57-66
6	4.0	3.6	51-56
7	5.1	4.0	41-50
8	6.5	5.0	34-40
9	6.9	6.6	30-33
10	7.7	6.9	27-29
11	8.3	8.0	25-26
12	9.0	8.3	23-24
13	9.9	9.1	21-22
14	11.0	10.0	19-20
15	12.4	11.1	17-18
16	13.2	12.4	16
17	14.1	13.2	15
18	15.2	14.2	14
19	16.4	15.2	13
20	17.9	16.5	12
21	19.7	18.0	11
22	21.8	19.7	10
23	24.5	21.9	9
24	27.9	24.5	8
25	32.3	27.9	7
26	38.5	32.4	6

Optimum operation was determined to be obtained when an 8-to-1 time compression was used. This was achieved by operating the Honeywell 7600 at 15 IPS playback speed (all data recording was accomplished at 1-7/8 IPS). The CAT then sampled 400 points at a rate of 3.2 kHz for an equivalent real time sampling rate of 400 samples per second.

The overall data playback/digitization system used for Experiment II is shown in Figure 18.

In order to simplify the search for useful data epochs, the data playback system needed a search control module which could be set to stop the analog reproducer when either a TNT stimulus solenoid signal was present or when a neutral stimulus signal was present as the search operator wished. Consequently, the stimulus presence detectors and the search control unit was designed to offer any signal option to the operator, including cessation of search at a preset time code. Since the quantity of data was staggering in the raw analog format, this control module was an invaluable adjunct to final data analysis.

Prior to each data digitization session, the playback device was calibrated by use of the standard signals included on each data tape. These signals allowed for both d.c. offset adjustment and for a.c. calibration, so that at the time of playback, the output of the data playback system was an exact replica of the input data derived from the EEG preamplifiers. Close control of d.c. offset not only allowed for the most linear operation of the playback amplifiers in the tape systems, but it also greatly simplified the process of digitization.

System digitization bandwidth was set by the Rockland analog filter prior to A/D (analog-to-digital) conversion. The overall system was found to be reliable and required no adjustment or repair throughout its service period. Indeed, the overall performance of the playback/digitization system of Experiment II greatly exceeded the requirements imposed by this research.

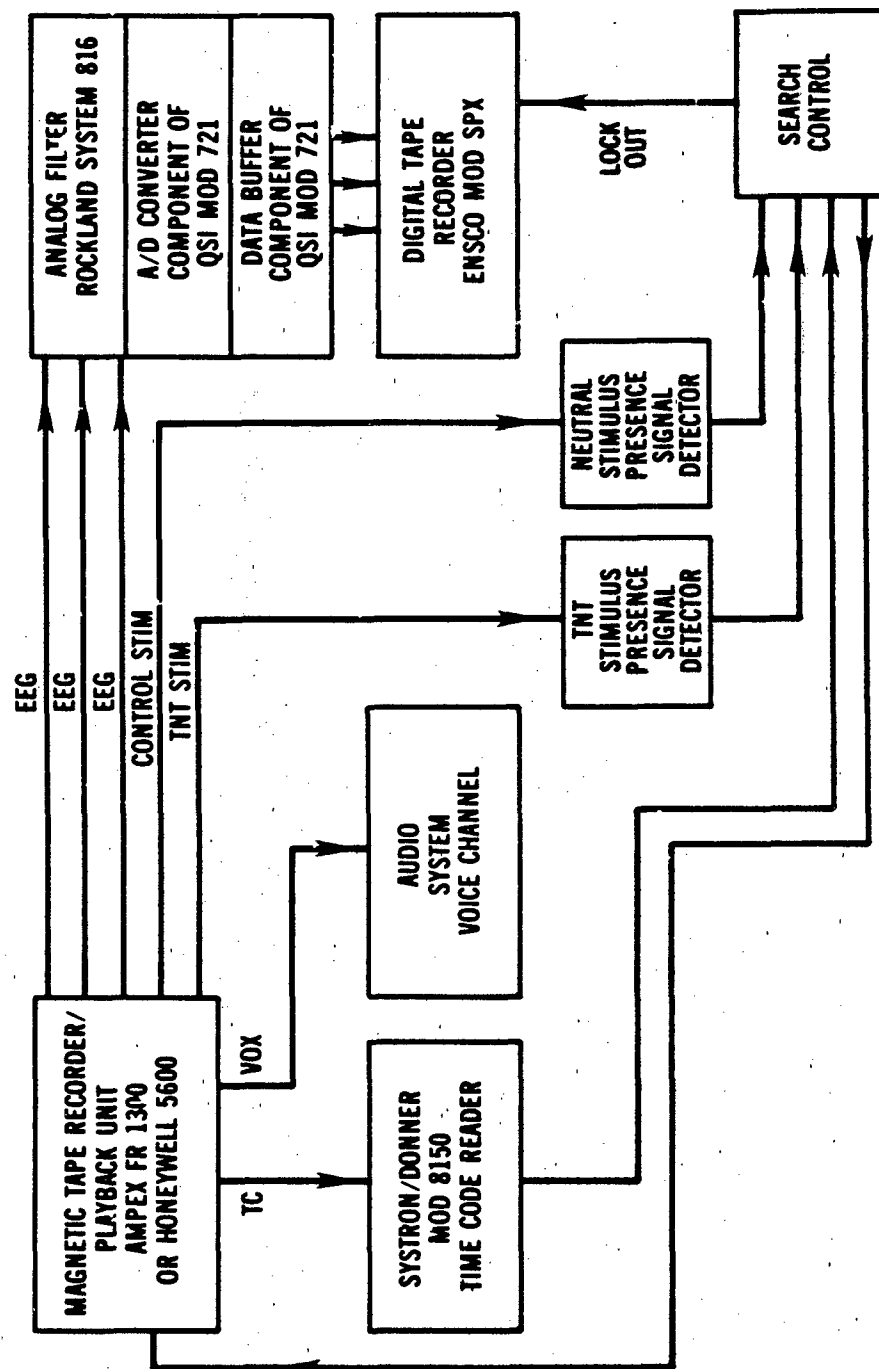


Figure 18. Data digitization system: Experiment II.

III. RESULTS

23. Summary of Results. It is evident that each of the four Theses which comprise this report have been proven beyond reasonable doubt. Thesis A postulated that laboratory rats were capable of identifying the odor of military grade TNT in the presence of olfactory clutter; Thesis B argued that if A were true, then the subjects could be induced by the methods of operant conditioning to signal the presence of TNT vapor and to refrain from signaling the presence of other (neutral) olfactory stimuli.

While a proof of Thesis A was initially suggested by the observation of unique behavioral changes which occurred in test subjects only when TNT vapor was present, much more definitive proof was achieved when Thesis B was verified. Hundreds of hours of preparation and testing were involved in this seemingly simple set of proofs, and the results are beyond question. It has been shown that laboratory rats (Sprague-Dawley strain) can, indeed, identify the presence of minute quantities of TNT vapor and that they will eagerly signal this identification by means of a bar press.

Thesis C was proved by the expedient of complete standardization of all training procedures and by strict adherence to this protocol. Thesis C proposed that more than one subject can be trained simultaneously by a single human operator whose sole function is to initiate the automatic program test sequence after placing the subjects in the test apparatus. It is certain that large numbers of rats can be trained to detect and announce the presence of TNT by automatic methods requiring the attention of only one laboratory technician.

The proof is of great importance to the pragmatic aspects of various biosensor programs, some of which have heretofore suffered defeat and elimination because of training costs alone.

Thesis D predicted that an event must occur in the cortical EEG as a direct consequence of the arrival of an olfactory stimulus which had heretofore resulted in the delivery of an extremely desirable reward. This event is referred to as an Anticipatory Evoked Spectral Change in the cortical EEG signals. The event was postulated to occur in a short time window immediately following the stimulus presentation, during which window the test animal would anticipate—from prior classical conditioning—the delivery of the pleasure reward which it so eagerly desired.

The proof of Thesis D required extensive data analysis employing sophisticated digital computers since this protocol was, ultimately, the only practical method for elucidating the presence of detection signals heavily masked by the neural "noise" of the background EEG. Thesis D has been shown to be valid; unique EEG signal features appear following exposure of the subject to low concentrations of TNT vapor.

Many years of intensive experiment lie between this initial proof of Theses A, B, C, and D and the realization of a feasible explosives detecting biosensor such as described earlier, but the rationale for expending this time and effort now exists in this report.

24. Behavioral and EEG Data. The results presented below are based upon the data collection of two major laboratory experimental efforts which consumed a period of about 3 yr. In all, over 1300 data epochs were examined in varying degrees of detail, and this quantity of data possibly would represent experimental excess, were it not for suspected errors in early data which were resolved only after all data acquisition was completed.

The presumed errors were related only to the procedures designed to verify Thesis D. At no time was there reason to doubt those experimental procedures which were implemented to verify the critical Theses A, B, and C. However, to preclude controversy, the entire experiment was implemented twice to the extent necessary to unequivocally argue the validity of all experimental data. In the interim period between the first experiment and the second experiment, the entire data-taking process was reviewed for competency and was extensively revised.

The rationale for expending this total amount of effort stemmed from a probably overcautious evaluation, wherein it was concluded that laboratory noise artifacts had possibly contaminated the first data applicable to Thesis D, in spite of the exercise of due caution. Also, there was some concern that the initial experimental bandwidth of 50 Hz was inadequate since there was no a priori reason to believe that some significant EEG signal components did not exist at 200 Hz or beyond. Since it was impractical to review the entire continuum of analog data from the first experiment through its many hours of background EEG, immediate pre-stimulus EEG, and voice channel comments, it was decided to rerun the experiment using extended bandwidth and more elaborate noise elimination techniques.

Examination of the expanded bandwidth second data set analog and digital tapes by spectral density measurements demonstrated that the most significant energy spectra were positioned below 40 Hz. This result was not really unexpected, since human EEGs have revealed the same distribution in records reported in the literature. Of 818 data epochs examined from the second data set, less than 10 revealed any evidence of significant EEG signal energy in the frequency region above 100 Hz, and these few instances probably were random occurrences, since the events do not routinely repeat in the subject during each data epoch (for a single type of stimulus) for that subject. No evidence of significant energy was obtained above 200 Hz in any epoch examined.

Since Experiment II was instrumented to allow for a data bandwidth ranging from d.c. to 300 Hz, it would have been necessary to digitize at a sample rate of 1024 Hz (to satisfy the Nyquist criterion) if full bandwidth digitization were required. Considering the amount of data on the analog tapes, the 818 analog data epochs (each ranging from 6 to 10 s in duration) would have consumed an inordinant amount of digital recording tape (even at 1600 bits/in. data density) and, consequently, machine time during analysis. Thus, prior to full-scale data digitization, a trial digitization was begun using full bandwidth. A small section of several data tapes was examined to determine if a reduced bandwidth of 200 Hz would be adequate, since this would halve the digital recording tape requirement by allowing sampling at 512 Hz at 1600 bits/in.

The EEG spectral density, or power spectrum, was derived from the digital data of Experiment II through the use of the procedure outlined below. A sample of the total data is $X(t)$ defined over the period $t_1 \leq t \leq t_2$ as depicted in Figure 19. Taking for Fast Fourier Transform (FFT) of the data sample results in the relationship:

$$F(\omega) = a(\omega) + ib(\omega) \quad (1)$$

where

$a(\omega)$ = the real component of ω

and

$b(\omega)$ = the imaginary component of ω .

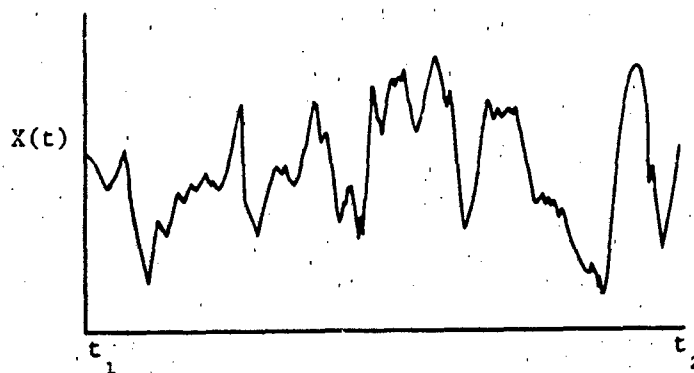


Figure 19. FFT relationships.

The power spectrum is then derived as the summed squares of the absolute values of $a(\omega)$ and $b(\omega)$:

or
$$P(\omega) = a^2(\omega) + b^2(\omega). \quad (2)$$

This expression is a real number representing relative power at any frequency in the sample spectrum. A plot of this function expressed as amplitude vs. frequency for 12 300-Hz bandwidth data samples is shown in Figures 20 and 21. Figure 20 depicts the calculated power spectrum for three samples of pre-TNT stimulus EEG data and three samples of post-TNT stimulus EEG data. Figure 21 shows the same calculation plots for pre-neutral and post-neutral olfactory stimuli data.

After several test runs, the plot bandwidth was reduced to the range of zero to 64 Hz, as shown. Similar plots of power spectra were made for other subjects and the overall results showed the same general variations from epoch to epoch. The data for the figures were chosen at random from these runs. The subject chosen was Rat B, the regimen was his third session, found on analog data tape 2A (Experiment II).

It is evident from these plots that there are no significant spectral components above 40 Hz in these data, and there is little evidence of significant signal power levels above 30 Hz.

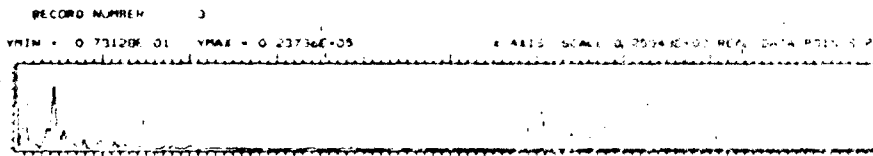
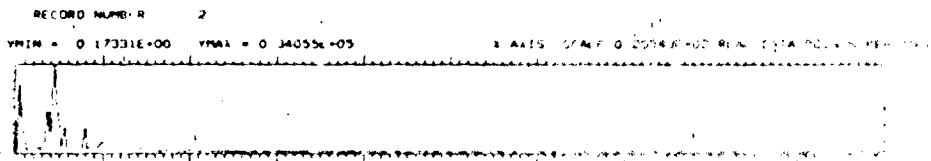
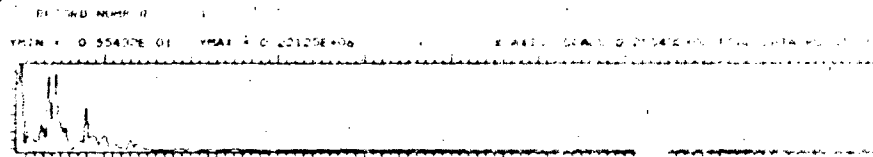
Considering the results obtained above and equating the cost of utilizing 300-Hz bandwidth in digitizing, the data playback system bandwidth was reduced to 200-Hz upper roll-off. Digitization then proceeded at 512-Hz sampling rate with 1600-bits/in. data density for all data epochs.

This verification of the appropriate bandwidth to be found in most, if not all, EEG samples greatly enhanced confidence in the integrity of the data set from Experiment I which, as noted earlier, was set at 50-Hz roll-off in the analog recordings. In spite of this assurance, the data from the pioneer experiment were not used in proving Thesis D since the data from Experiment II bore no residual stigma of any sort.

The behavioral data upon which the proofs of Theses A, B, and C rest is contained in the numerous data sheets from both Experiment I and Experiment II, such as those shown in Figure 15, in numerous pages of laboratory notes, and in the collective memory of the laboratory team members. The latter data bank—fallible human memory—was used in a series of "blind quizzes" to verify the unified data depicted in Figures 12 and 22. (An astonishing degree of agreement was found to exist between the collective and sometimes temporally distant observed behavioral characteristics and that depicted in the smoothed data of these figures.) Since behavioral data are notoriously difficult to categorize, standardize, and record, this agreement served to amplify confidence in the proofs of Theses A, B, and C.

SCALE EXTENDS FROM 0 TO 64 HZ

PRE
EVENT



POST
EVENT

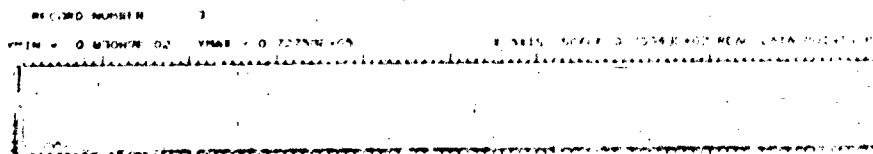
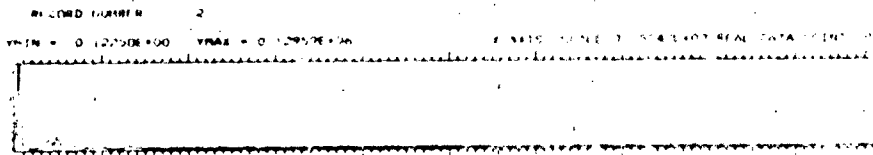
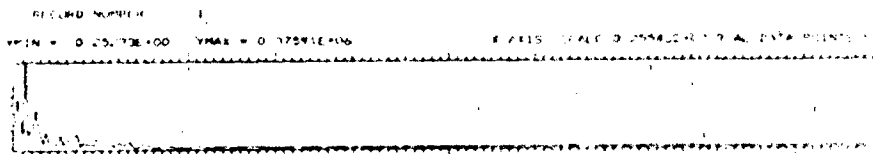


Figure 20. Rat B spectral density measurements TNT stimuli.

SCALE EXTENDS FROM 0 TO 64 HZ

PRE
EVENT

RECORD NUMBER 1
YMIN = 0.2700E+01 YMAX = 0.24130E+06 X-AXIS SCALE 0.2554E+02 REAL DATA POINTS PER INCH



RECORD NUMBER 2
YMIN = 0.3101E+00 YMAX = 0.8176E+05 X-AXIS SCALE 0.2554E+02 REAL DATA POINTS PER INCH



RECORD NUMBER 3
YMIN = 0.3242E+00 YMAX = 0.1116E+05 X-AXIS SCALE 0.2554E+02 REAL DATA POINTS PER INCH



POST
EVENT

RECORD NUMBER 4
YMIN = 0.5600E+00 YMAX = 0.1654E+07 X-AXIS SCALE 0.2554E+02 REAL DATA POINTS PER INCH



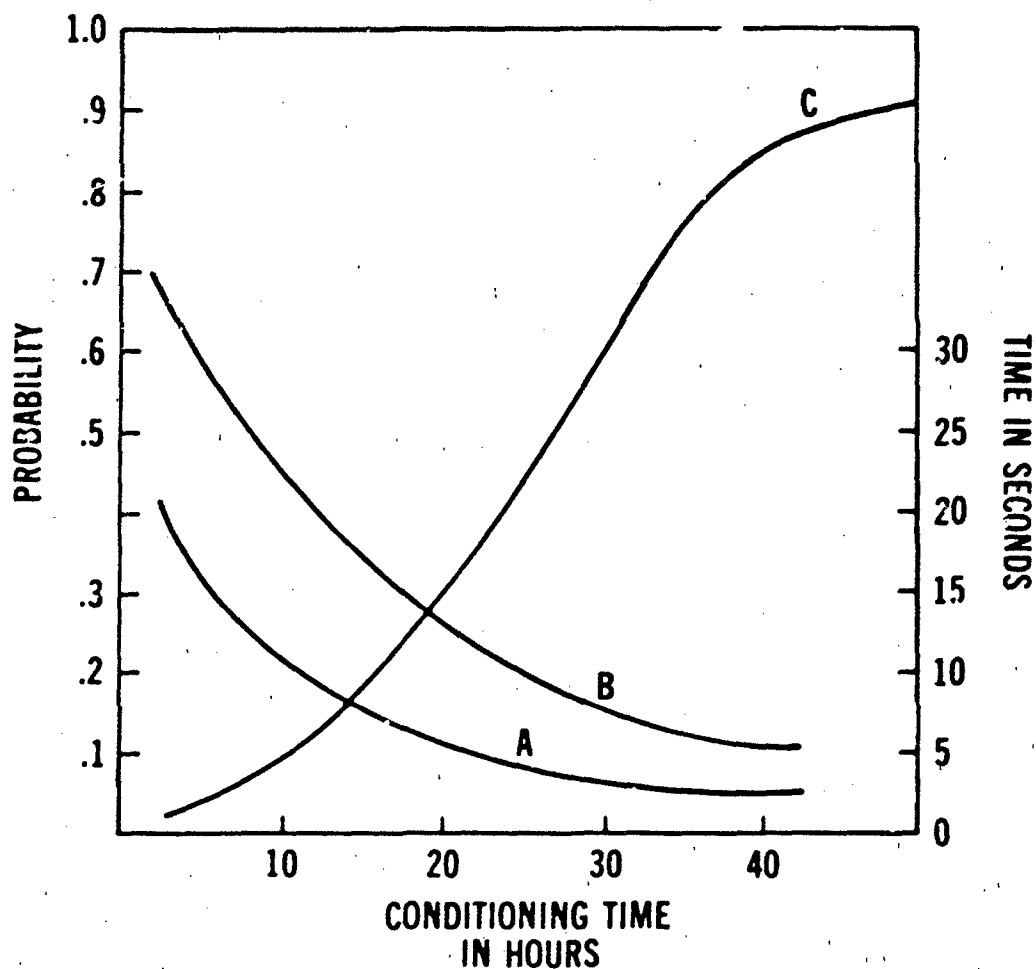
RECORD NUMBER 5
YMIN = 0.5000E+00 YMAX = 0.6172E+05 X-AXIS SCALE 0.2554E+02 REAL DATA POINTS PER INCH



RECORD NUMBER 6
YMIN = 0.2813E+01 YMAX = 0.1789E+05 X-AXIS SCALE 0.2554E+02 REAL DATA POINTS PER INCH



Figure 21. Rat B spectral density measurements neutral stimuli.



A = TREADLE PRESS TIME FOLLOWING TERMINATION OF ODORANT FLOW
 B = PROBABILITY OF FALSE RESPONSE
 C = PROBABILITY OF TRUE RESPONSE

Figure 22. Rat performance versus conditioning time.

25. Optimum EBS to Odorant Ratio. As described in detail earlier, some extinguishment of behavioral conditioning invariably accompanies any operational protocol in which a conditioned subject is rewarded each time a correct operant or classical response is effected in response to stimulus. Such behavioral response is termed habituation, and the negative effects, if unobserved or disregarded, can seriously alter the course of an experiment such as that under discussion. Further, these effects will probably be of indeterminate magnitude a posteriori, thus leaving an unknown degree of bias in the overall experimental results.

There is, in all creatures, a balance of emotion between habituation and anticipation which can be optimized by experiment, and such optimization is found by slowly varying the ratio of rewarding stimulus to detection stimulus. In the instant case, the frequency of availability of EBS was varied while the odorant density (olfactory stimulus level) was held as constant as possible.

Ten rats from Experiment I and five from Experiment II were studied for the effects of unity EBS/odorant presence ratio. Habituation was evident in those subjects which had passed the 40-h conditioning regimen (during which time a unity ratio was the rule) by about 20 h. Although the diminishment of the detection performance—as evidenced by treadle press—was highly variable between subjects, the average diminishment observed in all 15 subjects was found to be a drop to about 0.7 probability of detection. Since all the selected subjects had evidenced 0.9 or better probability of detection at the end of their initial 40-h conditioning regimen, this diminishment of performance was significant from the standpoint of practical application in explosives detection service.

As the EBS/odorant presence ratio was varied from unity to 0.3 by withholding EBS in a pseudo-random manner, the average subject began initially to increase his probability of detection (due to enhanced anticipation) and then extinguishment again appeared. The curve of Figure 12 represents the average performance of the test subjects. Note that there is a region, termed the "Zone of Maximum Anticipation," where the EBS/odorant presence ratio may be varied over rather wide limits (0.7 to 0.5) with little change in the observed detection performance. On either side of this zone, performance begins to deteriorate rapidly, especially as the ratio diminishes past 0.3. The data boundary point noted on the curve represents the limit beyond which it seemed inadvisable to go since the extinguishment of training would have been inimical to the experimental effort. The extrapolation (dashed curve) seems to be appropriate, but actual data were not obtained in the lower zone for any rat.

26. Verification of Theses A and B. Prior to this research effort, there were no known data which could unequivocally demonstrate that rats were able to detect TNT, even though assumptions were voiced to the effect that this capability *should* exist. It was essential, then, that Thesis A be proven valid at the earliest possible moment in the history of the research.

While Theses A and B are individual conceptual entities and verification of each was vital to the experiment, it became apparent early in the formulation phases of the experiment that Thesis A was most convincingly proved by the concurrent proof of Thesis B.

Thus, this phase of the experiment was designed to include the steps below:

a. The rat was shaped as described in Section II, "METHODS AND MATERIALS."

b. Treadle pressing resulted in EBS self-stimulus only when TNT vapor was present.

c. Optimal values for reinforcement/olfactory stimulus ratio were determined (Figure 12).

In compliance with this schedule, each subject was shaped until it was certain that the relationship between treadle press and the reward of EBS was firmly established. Table 4 shows a comparison of the (shaping) self-stimulating activity of 10 randomly selected rats. These data show that the subject animals clearly recognized the relationship between treadle press and the generation of the highly pleasurable EBS. The sessions enumerated in the right column were each approximately ½ h in duration with a maximum of five sessions permitted. Each subject was tested for maximal response in the first session by varying the stimulating signal current and the duration of the bipolar EBS signal described in Figure 5. Only those subjects which would respond to EBS signals in the range of 100 μ A to 500 μ A were considered useful to the program.

Table 4. Self-Stimulation Rates During Shaping

Rat ID	Treadle Press Rate Per Minute	Stimulus Signal Amplitude (μ A)	Stimulus Signal Duration (ms)	Session Number
A	63	300	300	4
B	187	250	300	4
C	64	250	250	4
G*	44	200	550	3
S	76	250	250	3
97	64	300	200	2
98	106	200	200	2
100	170	200	150	2
103	110	200	150	2
104	170	200	150	2

* Rat G was, at this point, a marginal performer, but at the end of the fifth and final session, he achieved the minimal requirement of 60 treadle presses/minute.

The rationale for the choice of these limits was:

d. 100 μ A was believed to represent the lowest signal current which could result in a reliable stimulus to the appropriate structures in the Medial Forebrain Bundle in view of the subject-to-subject anatomical variations and considering the signal "scattering" resulting from the volume conductor nature of the brain tissue.

e. 500 μ A represented the upper limit of safety from the standpoint of tissue destruction resulting from heating effects and from electrophoresis; further, it was known from earlier experiments that signals much in excess of 500 μ A could result in excitation of neural structures which cause behavior radically different from that desired (see paragraph 14, "EBS Signals").

Ultimately, 300 μ A was chosen (prior to formal data runs) to be the optimum stimulus current, and this level was held constant in all data runs in both Experiment I and Experiment II.

Similarly, the duration of the EBS signal was varied in initial testing and only those subjects responding maximally to stimulus durations ranging between 150 ms and 300 ms were chosen for further testing. It is important to note that stimulus duration is *not* defined as the pulse width of the stimulating signal which was held constant at a pulse group width of 600 ms; rather, stimulus duration is defined as the pulse train width. Since the pulse group repetition rate was set at 100 Hz, a 300-ms signal consisted of 30 bipolar pulse groups.

This duration was set at 200 ms (or 20 pulse groups) for the stimulus periods between EEG data taking sessions, and thus, a "standard" EBS was defined.

In addition to the limits imposed on the acceptable range of EBS signal parameters, the rats were required to attain a threshold rate of 60 treadle presses per minute at the end of the fifth half-hour session. Those subjects which did not reach this level were eliminated from further experimental service. Rat G was in jeopardy at the end of his third session, as evidenced by his press rate of 44 per minute. By the end of his fifth session, however, he achieved the required rate of 60 per minute.

It is of interest to note that the self-stimulus rate variation of nearly four to one (in the sample group shown in Table 4) in these early sessions did not represent any predictive index of performance as far as detection probability was concerned for those subjects which survived to the final phase of the experiment. Data of the type shown in Table 4 were essential, however, in verifying that correct placement of the EBS electrode had been achieved during surgery. Clearly, this was the situation for all subjects shown, although Rat G was a marginal case, especially in view of the 550-ms EBS dura-

tion necessary to cause this subject to stimulate at even 44 treadle presses per minute in his third session. Rat G represented the lower limit of acceptance of the group 20 subjects surgically prepared for the second EEG experiment, while Rat B represented the upper limit of performance of this group. In spite of his low self-stimulus rate, Rat G was an excellent detector of TNT, and he manifested evidence of extreme pleasure upon receiving EBS. Further, he tended to sniff at rates in excess of 9 sniffs per second when TNT vapor was present as compared to the group average of about 6 sniffs per second. (Normal breathing rate in the absence of TNT was generally about 1 rate per second in all test subjects.) A high sniff rate was usually present in those animals which seemed from observations of behavior to anticipate the pleasure of EBS to the greatest extent.

When a subject such as Rat G or B had shown interest in self-stimulus, the animal was then available for use in proving Theses A and B. This regimen began by placing the subject in a Faraday Box restraint in such a manner that he had access to a self-stimulus treadle. After accommodating to the restraint (Figure 4), the subject soon began to self-stimulate as he had done in the more amicable environment of his shaping Skinner Box (Figure 11), and finally all subjects appeared to be almost oblivious to the initially obnoxious restraint device.

The subjects were allowed to self-stimulate for about an hour at a 1.0 treadle press/EBS ratio, and then an automatic sequence was begun in which the EBS reward was present only when TNT vapor was delivered to the test box.

Figure 22 shows the greatly smoothed response of 10 randomly chosen subjects to this environment. Initially, an average subject tended to press the treadle at a rather high rate, but unless a press occasionally coincided with an "EBS ON" mode in the sequence, he soon lost interest in the treadle. After a few successes in obtaining EBS, however, the interest was renewed as evidenced by extended high-rate pressing for up to 15 s or 20 s after EBS was discontinued (Curve A). After about 30 1-h sessions, this extended post-EBS pressing had nearly disappeared, which implied that the subject had most probably begun to recognize that EBS would be forthcoming only when TNT vapor was present in the test chamber. At the end of the fortieth 1-h session, the average subject had reduced his false signaling probability to about 0.10, and he had brought his true alarm signaling probability to about 0.85 or better, as shown in Curve C.

The foregoing description greatly simplifies the regimen required to achieve the final test sequence which was derived after many trials with 40 rats during a period of nearly 2 yr. The reader is exhorted not to view the simple denouement as a measure of the difficulty of the experiment. Numerous variants of the final procedure were attempted. For example, the session length was varied from 15 min to 150 min; the EBS duration was varied; the restraint was altered or even eliminated, and so on through numerous

iterations of test parameters. Toward the end of these preliminary trials, the only variables which remained unresolved were session length and olfactory stimulus intensity. It was decided that a change in odorant intensity was not advisable at any point in the formal proof of Theses A and B, since it introduced an unnecessary factor which yielded no useful results in this research effort.

Thus, the procedure for verifying Theses A and B was derived and applied to the test groups in each of the overall experiments as noted above. At the end of a 40-h test sequence, the average subject clearly verified the Theses by his behavior. In the absence of TNT vapor, the subject generally ignored the treadle after about 30 h of conditioning, except for an occasional random press (perhaps this represented an attempt to hasten EBS, or just "to see if anything good would happen"). When TNT vapor was present at the 40-h point, the animal began furiously to press the treadle in an attempt to extract the maximal pleasure from what it may have come to recognize as an ephemeral event. As noted earlier, there was approximately a 500 ± 70 -ms transit time during which the TNT vapor bolus leading edge traveled from the solenoid valve to the test chamber. By using the solenoid energizing signal as an oscilloscope trigger, it was always possible to observe the first treadle press signal and thus a measure of latency was obtained. It is not possible to accurately define true physiological latency in this manner, however, since uncertainty in odor bolus transit time was so great, and apparently the animals occasionally were subject to spells of initial inattention—as are humans in long, boring waits—and this factor possibly could add several milliseconds to the apparent latency. However, it was always observed that the first treadle press was achieved within 1 second of the origin of the trigger. Considering also that the concentration of the odorant bolus leading edge was probably lower than that at the center, some indeterminate delay in signaling may have been due to an inadequate olfactory signal or diurnal variations in olfactory sensitivity. In any event, the response was always rapid when TNT vapor appeared, and the treadle press rate usually approached that achieved in self-stimulus sessions during the final shaping experiments. For example, Rat B was observed on several occasions to be pressing at a rate of 170/min during the 10-s "TNT ON" time which corresponds well with the rate shown in Table 4 for his fourth self-stimulus session.⁸³

⁸³ No formal data relating to press rate were accumulated after each animal had achieved at least the required minimum rate of 60/min within five training sessions, since these data were subject to some degree of diurnal variation and were of no foreseeable value to the experimental protocol or results. On occasion, each animal was placed in a Skinner Box and observed casually simply to assure the test operators that no regressive behavior patterns had become established. No significant (>20 percent) diminution of press rates were ever noted subsequent to final shaping unless several weeks of total inactivity had occurred between test sessions. No observations were made after classical conditioning was begun, since no treadle pressing was again allowed after this milestone was achieved.

The probability that the observed behavior is due to pure chance is small. It would have been necessary to apply various statistical tests (T-Test, etc.) had the results indicated signaling probabilities in the presence of TNT vapor of 0.50 or less, and signaling in the absence of TNT to be 0.30 or greater. Since these signaling levels were never observed in subjects which survived the first 10 h of a TNT/EBS pairing test, it was not considered necessary to resort to statistics for any type of assurance as to the validity of the observed results. It is significant to note that earlier work⁸⁴ with dogs resulted in much the same behavioral patterns only after several months of operant conditioning.

The realization that rats could be so rapidly and effectively trained was gratifying.

It is important to stress at this point that the exact nature of the olfactory cue was never a matter for investigation in this research. Certainly military grade TNT contains several substances in addition to 2,4,6-trinitrotoluene, but these substances are always present in approximately the same proportions in all samples of military grade TNT, and thus it is of no consequence to this research if the animal identifies one component or all components of this explosive, since detection of the aggregate substance is the desired experimental goal. Further, the matter of distractants was not pertinent, and hence this avenue of investigation was not explored in this research. It is evident from Army research with canines that certain substances (e.g., cocaine) applied topically to search areas can reduce the olfactory sensitivity to the vanishing point, but there would have been no merit in pursuing this matter during these experiments since the protocol sought only to prove that detection could occur and that this act could be made evident by the biosensor animal.

Similarly, no extensive effort was made to find the "ultimate" neutral stimuli since, in effect, any olfactory stimulus other than TNT was, by definition, neutral, and degrees of neutrality were of no interest. In the long term, the matter of distractants (or non-neutral, non-desired) stimuli will be of interest to the overall program of the sponsor, but such investigations were totally beyond the scope and intent of the instant program.

In summary, it was unequivocally shown by this experimental sequence that rats could in fact detect the odor of TNT vapor and that these mammals could be trained to signal reliably when they were aware of the presence of the TNT vapor. Thus Theses A and B were shown to be valid assumptions, and the procedures for inducing this conditioned behavior were entirely justified for continuing use throughout the remainder of the experimental effort.

⁸⁴ Nolan, R. V. and Gravette, D. L., "Mine Detecting Canines," Report 2217, U.S. Army, MERADCOM, Fort Belvoir, Virginia (1977).

27. Verification of Thesis C. At the conclusion of the test series which demonstrated the validity of Theses A and B, it seemed evident that Thesis C would be relatively simple to verify and, in fact, this was the situation. Using the conditioning regimen described earlier, five naive but behaviorally shaped subjects were chosen from the early population of animals used in Experiment II. Each test subject was given identical TNT/EBS pairings, thus stimulating sequentially what would have occurred simultaneously had sufficient laboratory facilities been available.

The learning patterns, while exhibiting variations due to the ever-present physiological and psychological differences in subjects, were, overall, similar to the general trends shown in Figure 22, and at the end of the 40-h training periods, all subjects were performing within limits of about ± 30 percent to the parameters of Figure 22. These limits compare favorably with the recorded performance on canines in earlier experiments.⁸⁵

There are several potential sources of error in applying sequential training to a simulation of simultaneous training, but the magnitude of these probable errors are of secondary importance when one considers the individual performance differences known to exist across the total subject population, even in the "clone-like" population of the Sprague-Dawley rats. One source of experimental error is the diurnal variation in relative humidity observed in the laboratory. Over the 5-day intensive test period used here, relative humidity varied between 60 and 80 percent with in-test variations of 5 percent or less, while the temperature of the ambient air was held to the range of 70° F to 75° F. These two environmental factors will result in some variation in the concentration of TNT in the odorant bolus delivered to the subject, and these variations could cause some variation in the detection performance of the subjects. No valid argument—pro or con—can be made for the magnitude of this physiological variance, since the present knowledge of the entire olfactory process borders on ignorance. Further, since it was always assumed that the odorant signal was well above the threshold for any normal subject, these small variations in odorant concentration are most probably insignificant as far as this research is concerned.

Of far greater significance is the diurnal variation in the psychological factors attendant to detection, and these effects would pertain regardless of whether the testing was sequential or simultaneous. Certainly rats exhibit daily changes in mood as do all mammals, but the rat, probably by virtue of its lesser intelligence, does not manifest the great swings in willingness to perform a given task, which is routinely observed in dogs (and humans). The psychological factors cannot be realistically evaluated in objective or quantitative terms. The collective observation of the research team indicated that a diurnal variance in a perceived "eagerness to work" of perhaps 5 or 10 percent occurred

⁸⁵ Nolan, R. V. and Gravitte, D. L., "Mine Detecting Canines," Report 2217, U.S. Army, MERADCOM, Fort Belvoir, Virginia (1977).

across all subjects. Since such observations cannot be given great scientific credence, the impact of psychological factors and observed behavioral minutiae were adjudged to be of marginal significance in the proof of Thesis C, especially since all research participants agreed that "little variation" was evident in individual subject performance throughout any 40-h test period. Since it was not possible to evaluate accurately the psychological status of any test subject at any time, one can only assume that any subject was operating (as a detector) at some "near mid-point" between its maximum and minimum limits at any given time. If this premise is acceptable, then the possibility exists for indeterminate unit-to-unit variations in performance, regardless of whether the units were tested sequentially or simultaneously, and therefore a sequential test program can indeed simulate a simultaneous test.

It is most important to note here that the continuously observed indifference evidenced by all test subjects toward man as an individual argues strongly in favor of the concept of automatic training, since even if the test operators were changed several times during training, there would be no resulting impact upon the depth of conditioning.⁸⁶

This final factor is of great significance not only to the immediate test results, but in consideration of future systems employing rats as biosensor detector elements, since the optimal biosensor animal must be one which requires no emotional ties to any human element in system operation.

Considering the arguments above, it is possible to state that the assumption of Thesis C is valid.

28. Verification of Thesis D. It became apparent through behavioral observations in the earliest phases of this research, that an emotional event of extreme intensity was induced by the arrival of the TNT vapor/air mixture at the nares of properly trained test subjects. Similarly, it was apparent that little or no excitement was manifested by the subjects when neutral olfactory stimuli were delivered. Even totally naive observers were almost immediately able to determine the nature of the applied olfactory stimulus by simply watching the gross behavioral changes which occurred following activations of the odorant delivery relays. Comparison of these "blind test" observations with a pre-planned stimulus delivery schedule demonstrated that—after perhaps 5 min of test observation—the observers could correctly state which relay activations caused TNT vapor delivery and which allowed a neutral stimulus to pass to the subject.

Emotional events of this magnitude sure must result from strong excitation of some undefined focus or foci in the brain of the test subjects. The most likely locus for these foci is in the structure of the limbic system, and one would therefore expect to find the strongest event-related potentials in the cingulate EEG electrode.

⁸⁶ To preclude even a suggestion of error in this concept during this research, only one test operator was employed in the experiment for Thesis C, even though three years of experience argued that rats are totally indifferent to which human is present and/or which human is handling them physically or operationally.

While all analyses were directed first to the ensemble of three electrodes, the final analysis ultimately was concentrated in extensive examination of cingulate electrode signals.

For the sake of historical completeness, the analytical methods which yielded minimal results will be summarily described first. Following these descriptions, the algorithms which successfully elucidated event-related spectral features will be discussed in depth.

a. **Pearson Product Moment Correlations.** In Experiment I, the raw analog were fed into the data system described in Section II. In this system an analog filter admitted frequencies between 1.0 Hz and 39.0 Hz (12-dB/octave roll-off) to a Schmitt trigger which, in turn, delivered a series of pulses which were the analog of the baseline crossings of the applied EEG signals (see Figure 17). Only signals from desired data epochs were allowed to pass through the gate in the signal stimulus detector.

The desired epochs thus delivered triggers to the Computer of Average Transients (CAT) which scanned through its addresses (at a 3.2-kHz rate) in the intervals between triggers. The digital processing in the CAT allowed for combining like addresses in the 26 EEG frequency bands shown in Table 3, thereby generating histograms of time-in-band versus frequency band.

The histograms so derived then represented the relative power spectral density of the data epoch under examination. Computations of this type were made for the following permutations of stimulus events:

Pre-TNT vs Post-TNT.
Pre-Neut vs Post-Neut.
Pre-TNT vs Post-Neut.

These data were then presented to a PDP-11/44 digital computer programmed to perform Pearson Product Moment Correlations upon these histogram data from the CAT. The correlations were made from the 26 data points in the power spectrum in the permutations shown above.

Briefly stated, the Pearson Correlation is described as:

$$\vec{X} = (X_1, X_2, \dots, X_n)$$

$$\vec{Y} = (Y_1, Y_2, \dots, Y_n)$$

Correlation between X and Y is accomplished by pairing

$$(X_1, Y_1), (X_2, Y_2) \dots (X_n, Y_n)$$

$$\text{Raw score: } R(X, Y) = \frac{\left\{ n \sum_{i=1}^n (X_i, Y_i) - \left[\left(\sum_{i=1}^n X_i \right) \left(\sum_{i=1}^n Y_i \right) \right] \right\}}{\sqrt{\left[n \sum_{i=1}^n X_i^2 - \left(\sum_{i=1}^n X_i \right)^2 \right] \left[n \sum_{i=1}^n Y_i^2 - \left(\sum_{i=1}^n Y_i \right)^2 \right]}} \quad (3)$$

$$\text{If: } \bar{X} = \left[\frac{\sum_{i=1}^n X_i}{n} \right] \text{ and } \bar{Y} = \left[\frac{\sum_{i=1}^n Y_i}{n} \right]$$

$$\text{Then: } \sigma = \sqrt{\frac{1}{n} \sum (X_i - \bar{X})^2} \quad (4)$$

$$\text{and: } Z_{X_i} = \left[\frac{X_i - \bar{X}}{\sigma} \right] \text{ and } Z_{Y_i} = \left[\frac{Y_i - \bar{Y}}{\sigma} \right]$$

$$\text{then standard score: } R(X, Y) = \left[\frac{\sum_{i=1}^n (Z_{X_i} Z_{Y_i})}{n} \right] \quad (5)$$

The general results are available from these data. From the CAT, the relative power spectral density between events can be examined, and from the Pearson Correlations, a measure of the statistical relevancy of the examined events may be obtained.

If the background EEG signals were statistically stationary during the period of a data epoch, then this technique would have considerable merit since it is sensitive to changes in the spectral density so derived. However, even in test subjects which are quiescent physically, the EEG is far from satisfactory even over a 1-s interval. Over an 8-s interval, such as was used in this early attempt, it was soon evident that the Pearson method cannot accommodate the background signal variations resulting from normal brain activity.

The data were examined for evidence of low correlation coefficient numbers which would indicate that a change in spectral density had occurred which was uniquely related to the stimulus event occurring in the post-stimulus data epoch. It was postulated that, when comparing epochs with high stationarity, high correlation coefficients would appear when a stimulus other than TNT occurred and low coefficients would appear when a TNT stimulus caused an anticipatory shift in the post-stimulus cortical EEG spectrum.

A glance at Tables 5 and 6 (which depict data from Experiment II) will show that this ideal situation does not exist. Table 5 shows a slightly higher mean value for the coefficients than does Table 6, and one is tempted to assign significance to the fact that there is a greater shift in the pre-event/post-event TNT coefficients than existed with pre-event/post-event neutral stimulus data. Tables 5 and 6 depict only brief samples of the data mass, but nothing of great significance was found by this method when over 100 data epochs were examined by the Pearson Correlation Method.

It was also tempting to ascribe significance to observed changes in each of the 26 EEG frequency bands, and, in fact, early in Experiment I it appeared that there was an increase in "normal" spectral density in bands 1 through 4 and bands 25 and 26 when, and only when, TNT stimuli were present. The T-test gave some indication that statistically, a change did occur, but, overall, the results were too nebulous to consider from the pragmatic credo which drove the experiment. For this reason, the Pearson correlation method was abandoned in Experiment I. It was tested again in Experiment II (as noted in Tables 5 and 6) and again there was no significant merit to the method, and thus this signal treatment approach was finally dropped from further consideration.

Table 5. Pearson Product Moment Correlations: Neutral Event
Session 2 (Analog Tape 2A)
Rat B
Pre-Event/Post-Event Correlations

Event No.	EEG Site*	Correlation
1	P	0.289
2	O	0.357
4	P	0.549
5	O	0.663
7	P	0.525
8	O	0.610
10	P	0.504
11	O	0.369
13	P	0.782
14	O	0.831
16	P	0.732
17	O	0.651

*P = Parietal

*O = Occipital

Pre-event = 4 s prior to olfactory stimulus.

Post-event = 4 s after arrival of olfactory stimulus.

Table 6. Pearson Product Moment Correlations: TNT Event
Session 2 (Analog Tape 2A)
Rat B
Pre-Event/Post-Event Correlations

Event No.	EEG Site*	Correlation
1	P	0.401
2	O	0.342
4	P	0.644
5	O	0.646
7	P	0.722
8	O	0.532
10	P	0.347
11	O	0.100
13	P	0.139
14	O	0.350
16	P	0.395
17	O	0.273

*P = Parietal

*O = Occipital

Pre-event = 4 s prior to olfactory stimulus.

Post-event = 4 s after arrival of olfactory stimulus.

b. Spectral Separator. In an attempt to locate the most applicable technique for identifying the presence of an unknown signal in noise, several organizations which were known to offer expertise in signal feature extraction techniques were contacted. A promising algorithm discovered during this search was the proprietary technique devised by ENSCO, Incorporated, of Springfield, Virginia, which was termed Spectral Separator. This technique was originated to measure spectral differences between two classes of events—with no a priori knowledge of either event—which was the presumed case with the EEG epochs.

Assume that there are n_a data segments for type A data, and that there are n_b data segments for type B data. Spectral Separator then functions as follows:

The power spectra of type A events is formed and averaged over all n_a samples:

$$P_a(\omega) = \frac{1}{n_a} \sum_{i=1}^{n_a} P_{a_i}(\omega) \quad (6)$$

where

i = i th record

ω = measure of frequency

also:

$$P_b(\omega) = \frac{1}{n_b} \sum_{i=1}^{n_b} P_{b_i}(\omega) \quad (7)$$

Then the variance of type A and type B events is calculated:

$$\hat{\sigma}_a^2 = \frac{1}{n_a - 1} \sum_{i=1}^{n_a} [P_{a_i}(\omega) - \hat{P}_a(\omega)]^2 \quad (8)$$

and

$$\hat{\sigma}_b^2 = \frac{1}{n_b - 1} \sum_{i=1}^{n_b} [P_{b_i}(\omega) - \hat{P}_b(\omega)]^2 \quad (9)$$

From these steps is derived the relationship:

$$S(\omega) = \frac{[\hat{P}_a(\omega) - \hat{P}_b(\omega)]}{[\hat{\sigma}_a^2(\omega) + \hat{\sigma}_b^2(\omega)]} \quad (10)$$

and $S(\omega)$ is termed the estimator or Spectral Separator.

Now assume that a new event—a new data record—becomes available, and its power spectrum $P_x(\omega)$ is formed. Combining $P_x(\omega)$ with $S(\omega)$ to form R :

$$R = \frac{1}{\omega \max} \sum_{\omega=0}^{\omega \max} [P_x(\omega) \cdot S(\omega)] \quad (11)$$

where $\omega \max$ = maximum frequency examined.

R tends to be small if the new event belongs to the set of type A, and tends to be large if the new event is of the set of type B.

Over a large number of events, a 'distribution normally' occurs wherein the values for R for type A events are separated from the grouping of values of R for type B events as shown in Figure 23. R_0 , the group intersection point, is chosen and the premise is made that:

- if $R > R_0$ then a type B event is likely;
- if $R < R_0$ then a type A event is likely.

The greater the separation of R values for types A and B events, the more dissimilar the classes of events. When the differences are blurred—that is, when values of R fall nearly equally on either side of R_0 for each class of events—then there is low probability that two distinct classes exist, and the events are therefore random occurrences.

This technique is especially sensitive to class differences since it searches only for spectral differences, and then it weighs these differences by their respective variances.

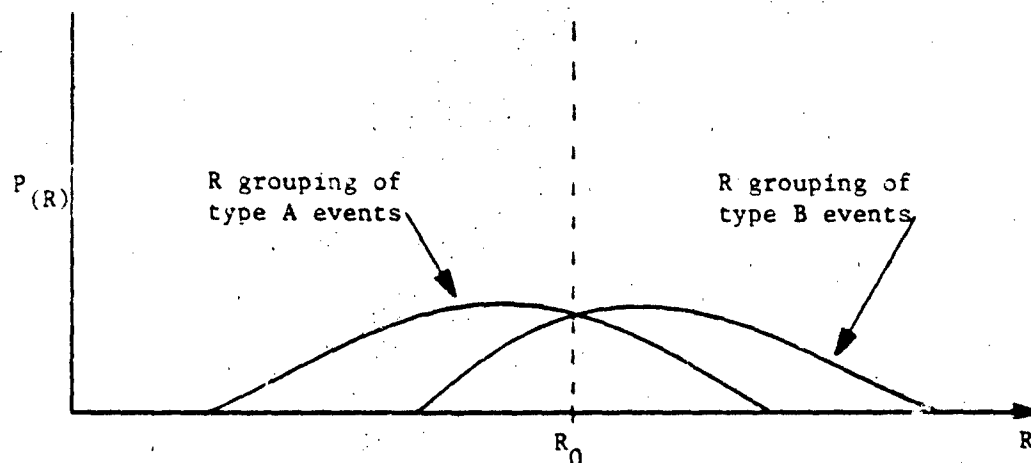


Figure 23. Spectral separator relationships.

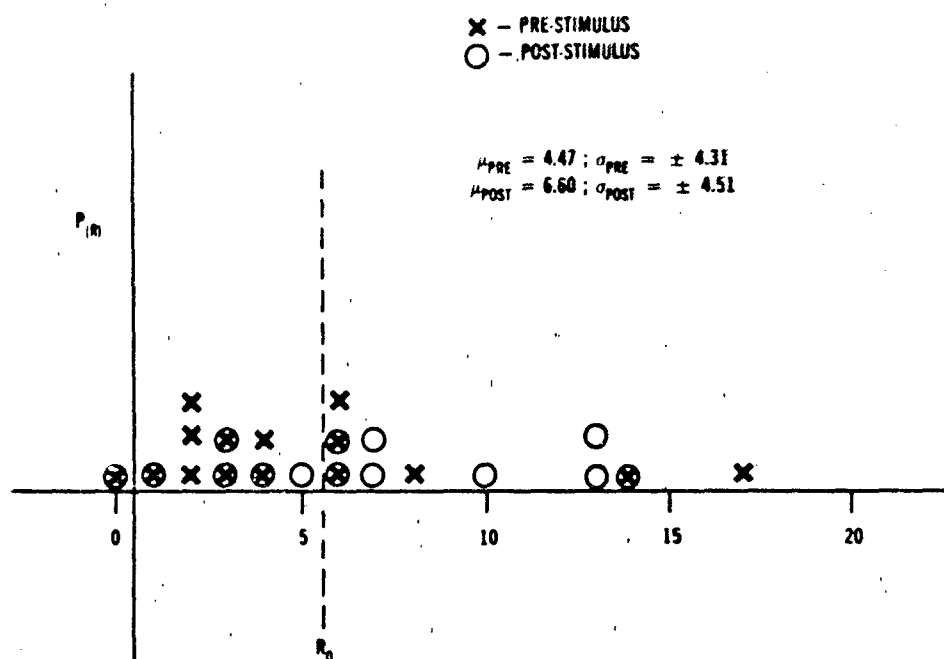
The plots of Figure 24 show the distribution of R values for a segment of data set 5A (for Rat G). It is tempting to examine such data and to visualize an envelope to the A and B groupings of R values, and indeed, Spectral Separator appears to show, in this instance, some separation of R values for both neutral and for TNT stimuli, but the difference is slight, and statistically there is no real evidence of a significant difference between R groupings for the two classes of stimuli, as is apparent from the values for the mean (μ) and for the standard deviations (σ). Thus the technique is of no value with the data available.⁸⁷

Failure of Spectral Separator did not preclude the existence of an AESC; rather, this failure implied that the spectral variation is either too random in structure or too low in amplitude (or both of these) for this method of detection.⁸⁸

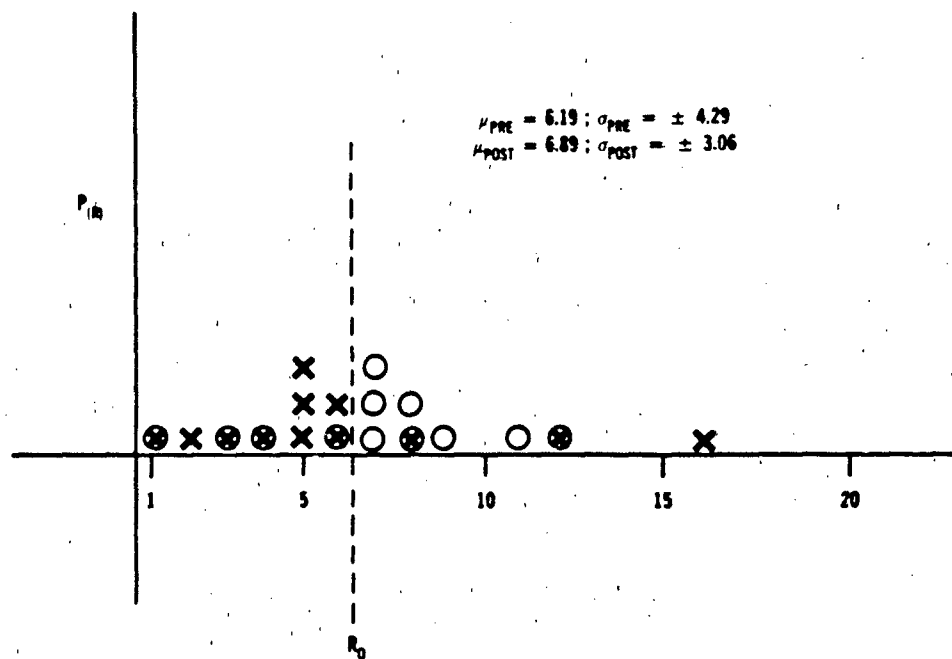
c. **The Covariance Process.** One of the two successful signal analysis processes used for the proof of Thesis D was an algorithm in the general form of the Covariance process. The process used may be described by the basic considerations below.

⁸⁷ The data of Experiment I were not tested since no reason was obvious for using those potentially less "clean" rats.

⁸⁸ Data derived from the successful analyses show that the signal is not random, merely low in amplitude.



NEUTRAL STIMULI



TNT STIMULI

Figure 24. Spectral separator: TNT and neutral stimuli.

If we let each of N sampled functions of time be represented as:

$$X_{k_{1,N}} = x_{k_1} \cdot x_{k_2} \cdot x_{k_3} \cdots x_{k_i} \quad (12)$$

where the mean of each function, taken over an interval from i to $i + \Delta$ is:

$$\bar{X}_{k_i}(\Delta) = \frac{1}{\Delta + 1} \sum_{j=i}^{i+\Delta} x_{k_j} \quad (13)$$

and the variance, over the same interval is:

$$\sigma^2_{k_i}(\Delta) = \frac{1}{\Delta} \left[\sum_{j=i}^{i+\Delta} (x_{k_j} - \bar{X}_{k_i}(\Delta))^2 \right] \quad (14)$$

By definition, the normalized average cross product of any two functions, K and H , is the correlation coefficient:

$$C_i(X_K, X_H, \Delta) = \frac{\sum_{j=i}^{i+\Delta} [x_{K_j} - \bar{X}_{K_i}(\Delta)][x_{H_j} - \bar{X}_{H_i}(\Delta)]}{\left\{ \sum_{j=i}^{i+\Delta} [x_{K_j} - \bar{X}_{K_i}(\Delta)]^2 \sum_{j=i}^{i+\Delta} [x_{H_j} - \bar{X}_{H_i}(\Delta)]^2 \right\}^{1/2}} \quad (15)$$

Since the normalization is with respect to the derivation, σ , we have:

$$C_i(X_K, X_K, \Delta) = 1. \quad (16)$$

Considering now all possible combinations of K and H in equation 4, the correlation coefficient matrix is configured as:

$$M_i(\Delta) = \begin{bmatrix} C_i(X_1, X_1, \Delta) & C_i(X_1, X_2, \Delta) & C_i(X_1, X_3, \Delta) & \dots & C_i(X_1, X_n, \Delta) \\ C_i(X_2, X_1, \Delta) & C_i(X_2, X_2, \Delta) & C_i(X_2, X_3, \Delta) & \dots & C_i(X_2, X_n, \Delta) \\ C_i(X_3, X_1, \Delta) & C_i(X_3, X_2, \Delta) & C_i(X_3, X_3, \Delta) & \dots & C_i(X_3, X_n, \Delta) \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ C_i(X_n, X_1, \Delta) & C_i(X_n, X_2, \Delta) & C_i(X_n, X_3, \Delta) & \dots & C_i(X_n, X_n, \Delta) \end{bmatrix}$$

Examination of the matrix reveals that it is symmetrical about the diagonal. It is then apparent that only half the matrix—excluding the diagonal—need be calculated.

From equation 16: $C_i(X_1, X_1, \Delta) = 1$

and $C_i(X_2, X_2, \Delta) = 1$ and so on.

From equation 15: $C_i(X_i, X_j, \Delta) = C_i(X_j, X_i, \Delta)$ and so on.

If Δ is termed the "sample window," then this N^2 matrix is defined for every sample, i . In order to simplify the inspection of the ensemble covariance, the terms of the matrix thus calculated are summed and averaged as:

$$A_i(\Delta) = \frac{(T M_i(\Delta) T^T - N)}{(N^2 - N)} \quad (17)$$

where T is a vector of length N (1, 1, 1, ...) (note that the diagonal is not included). A , then, has the property such that:

$$1 \geq A_i(\Delta) \geq -1$$

and A is zero for ensembles which do not covary.

In the Figures 25 through 39, the curves displayed are plots of $A_i(\Delta)$. The values of Δ , given in data points per second, are shown on each individual plot.

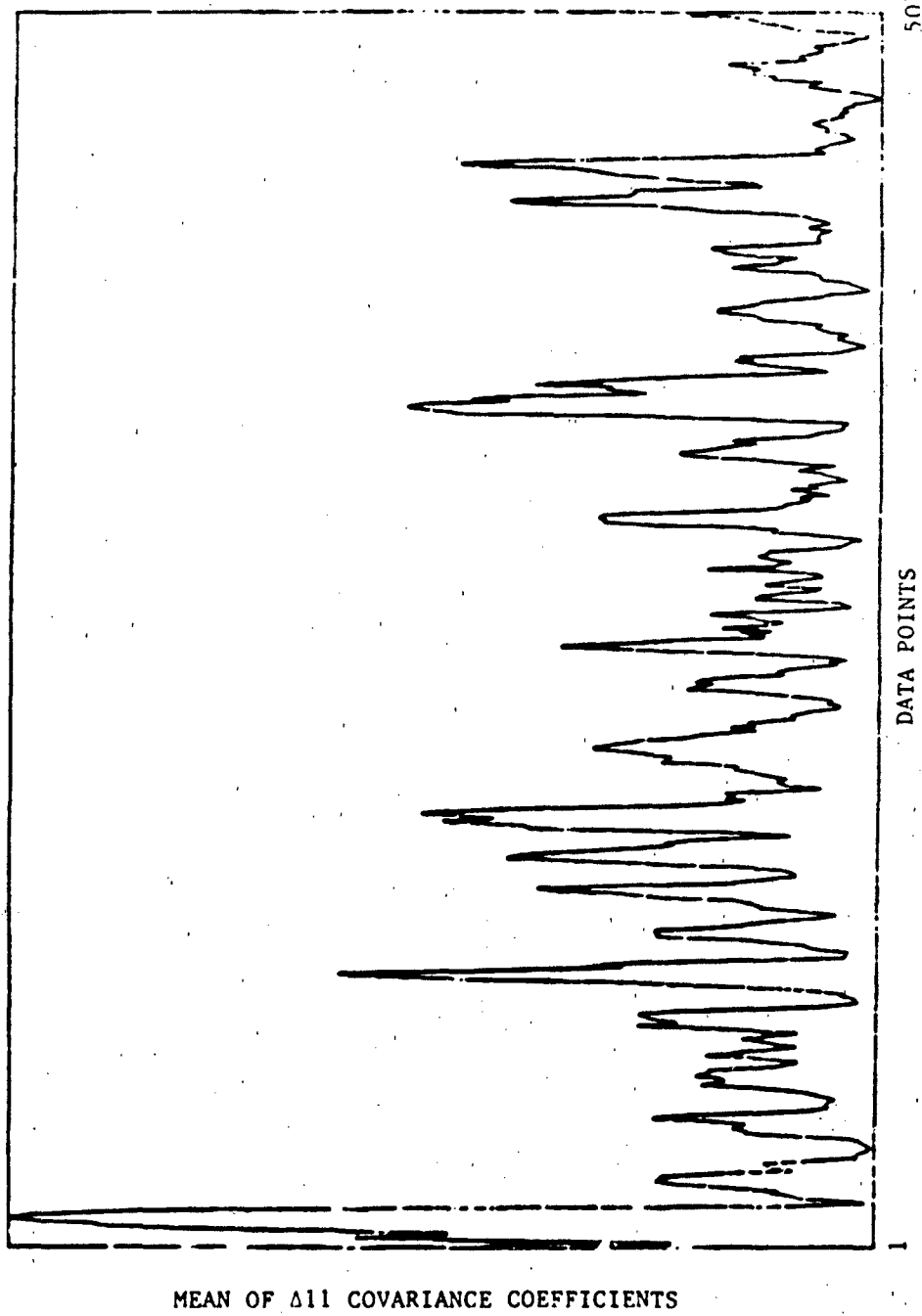


Figure 25. Mean of $\Delta 11$ covariance coefficients for interval $T\phi$ for 25 data epochs, Rat C.

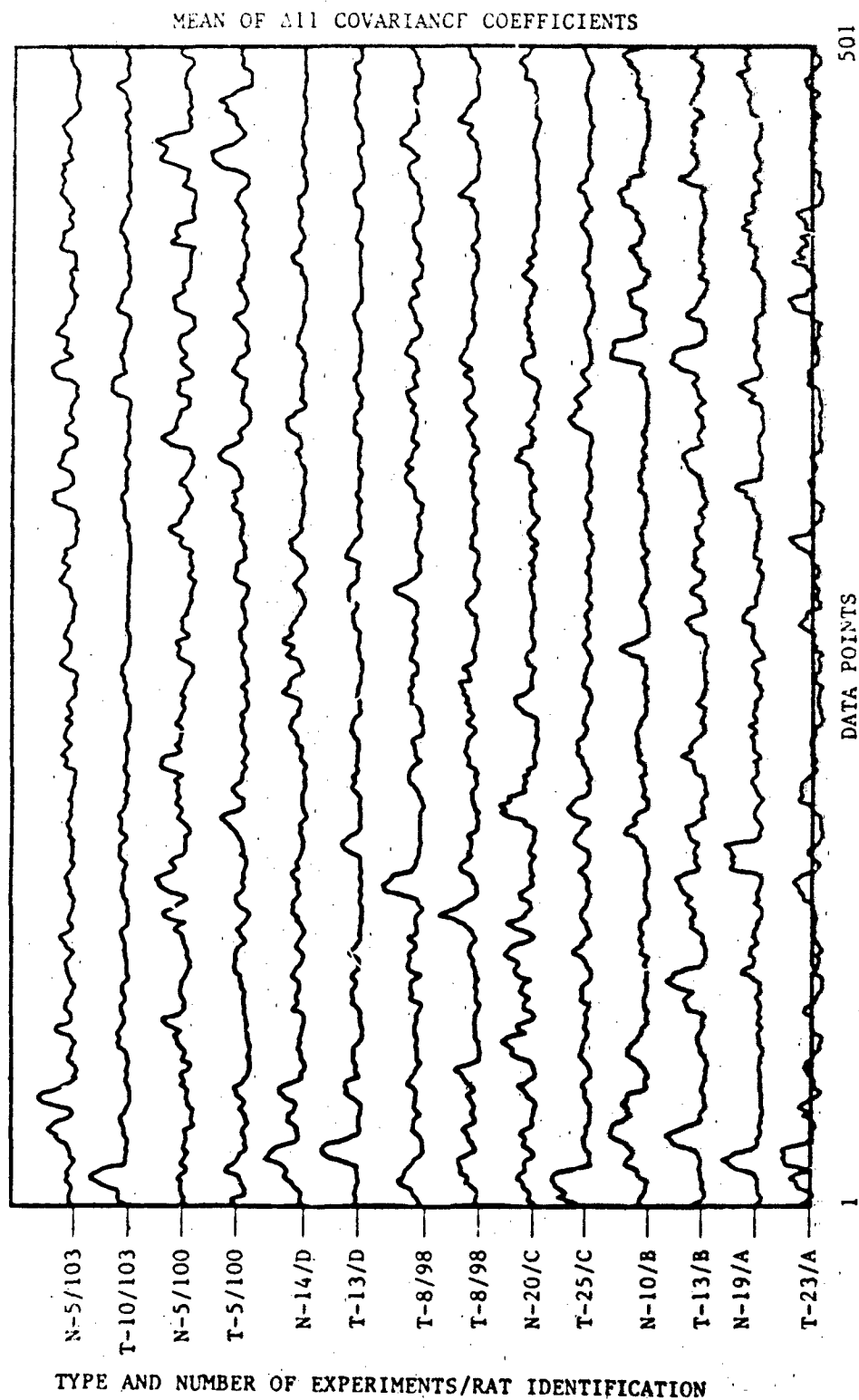


Figure 26. Mean of normalized covariance coefficients for neutral and TNT stimuli; 165 experiments across all rats.

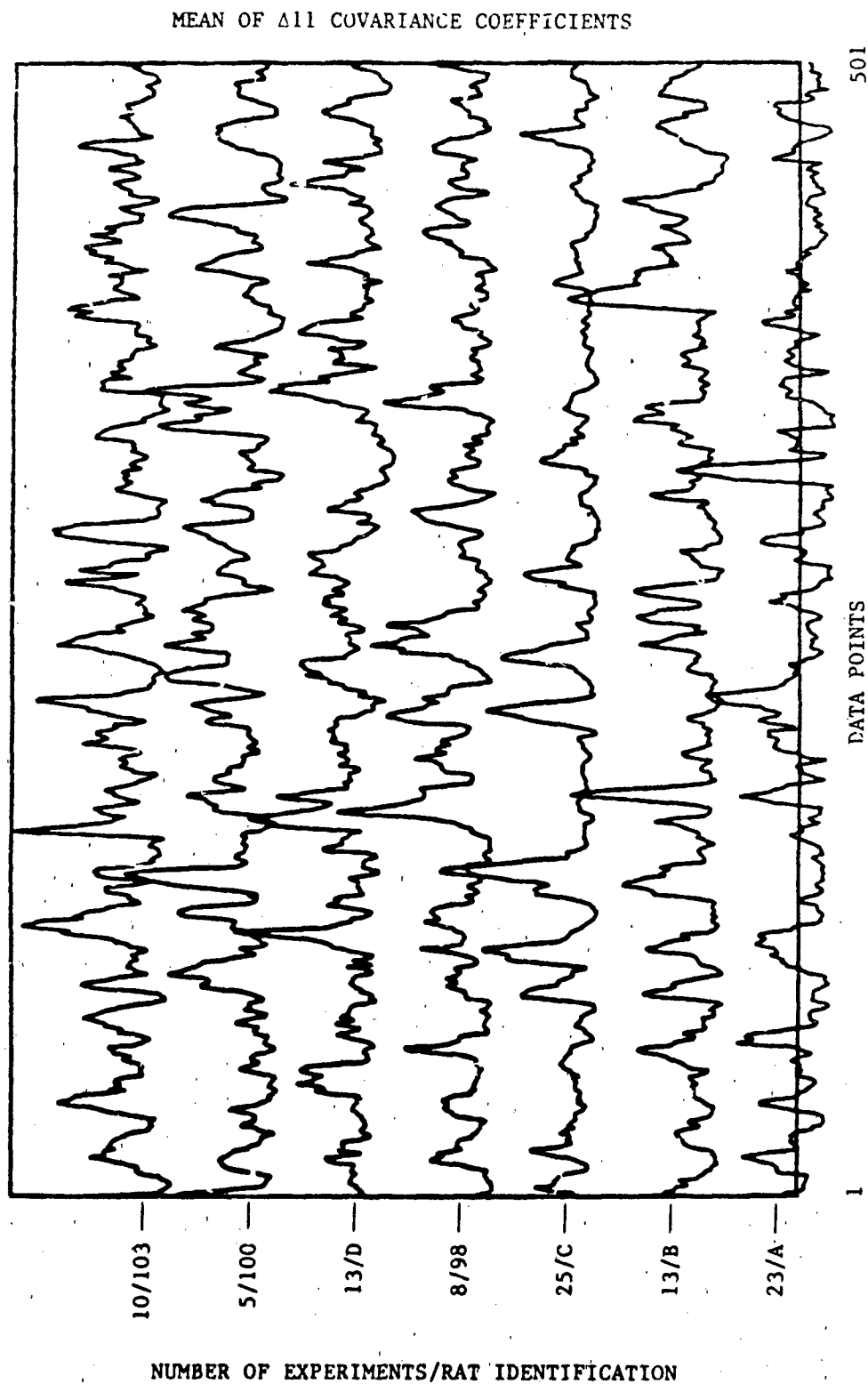


Figure 27. Normalized $\Delta 11$ covariance means by rat during interval T-1.

MEAN OF $\Delta 11$ COVARIANCE COEFFICIENTS

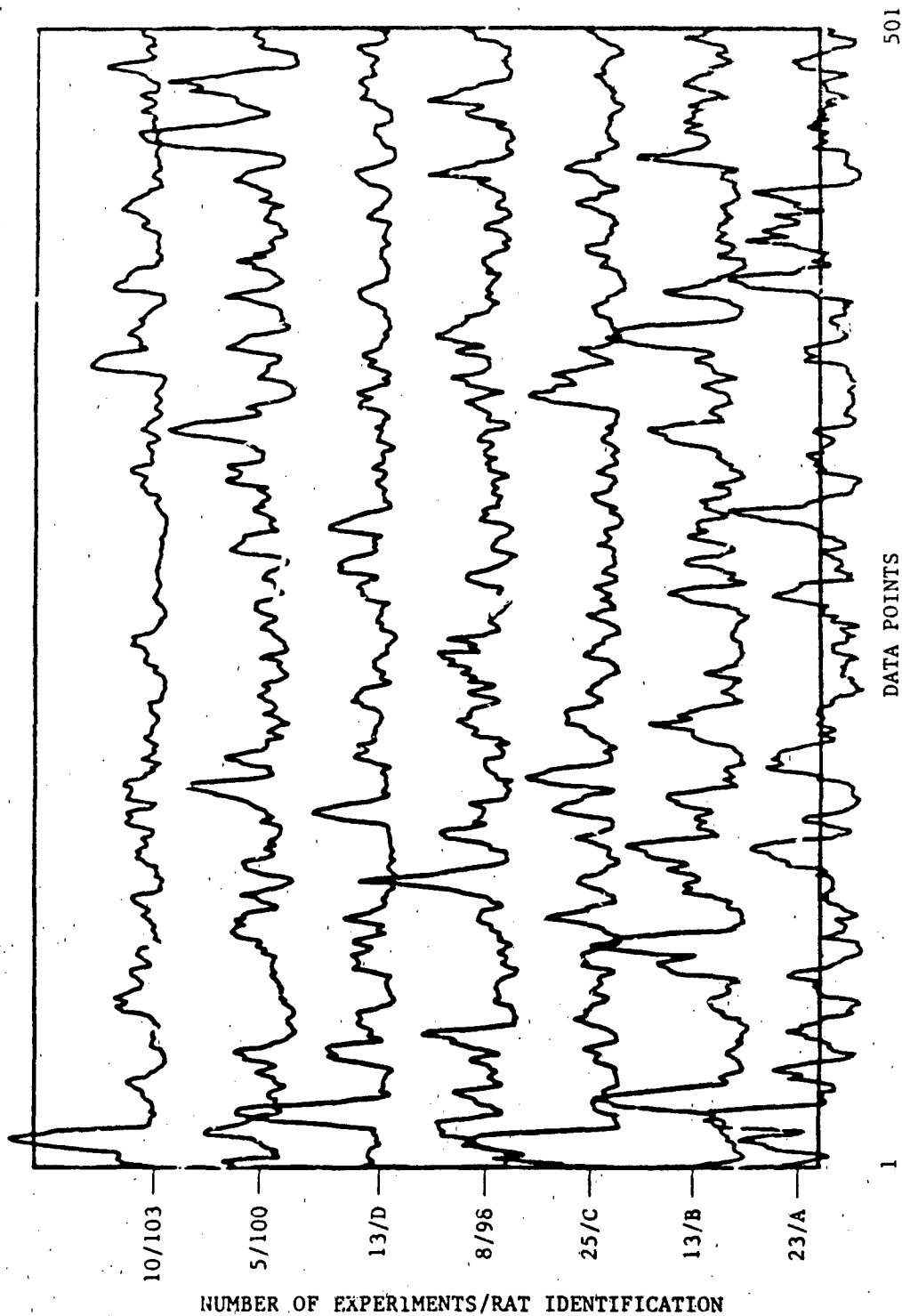


Figure 28. Normalized $\Delta 11$ covariance means by rat during interval T_0 .

MEAN OF ALL COVARIANCE COEFFICIENTS

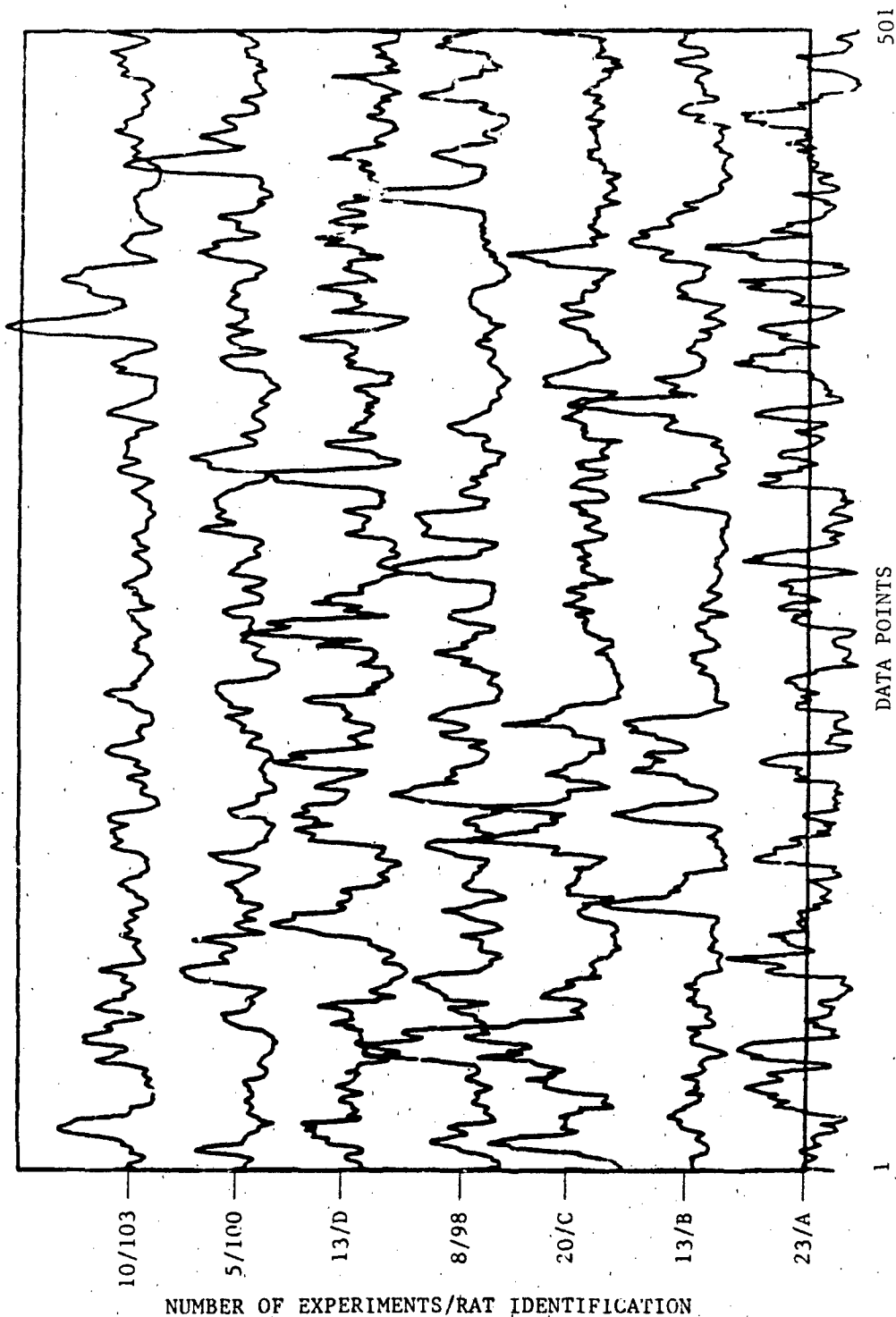


Figure 29. Normalized $\Delta 11$ covariance means by rat during interval T+1.

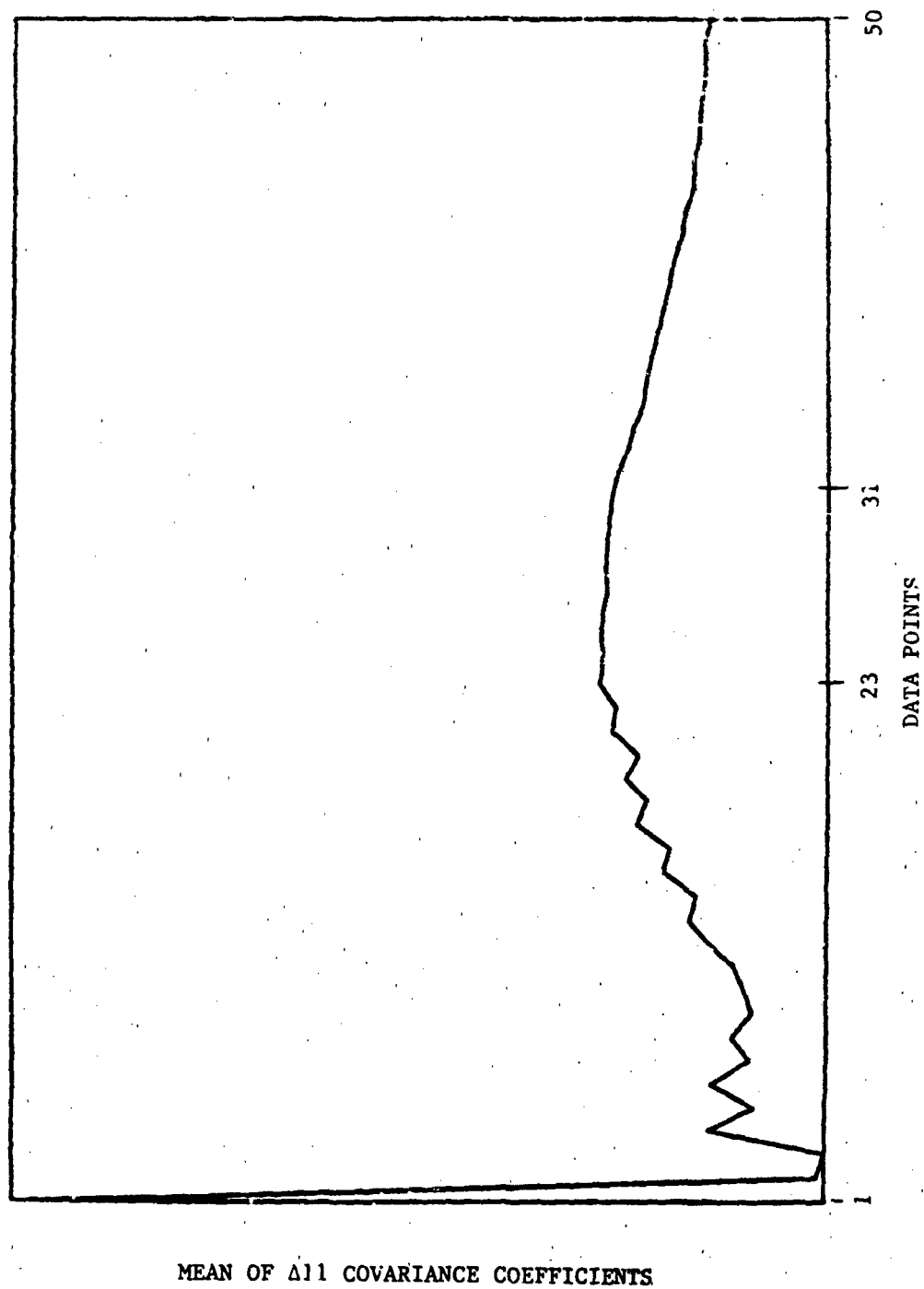


Figure 30. Mean of covariance for Δ_{11} covariance means at time 220/512 in $T\phi$ as a function of Δ .

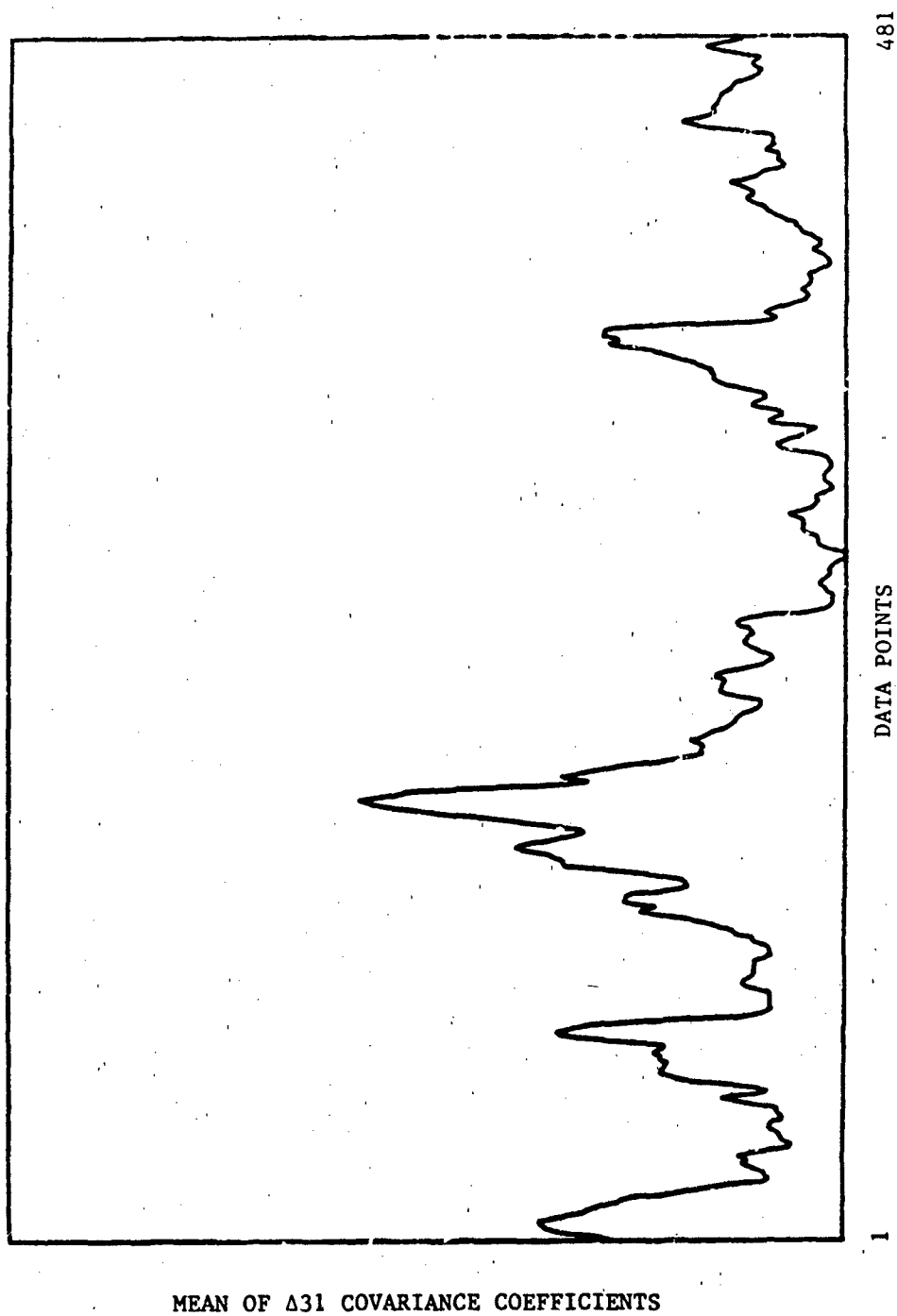


Figure 31: $\Delta 31$ covariance mean of $\Delta 11$ covariance means of individual rats during interval T-1.

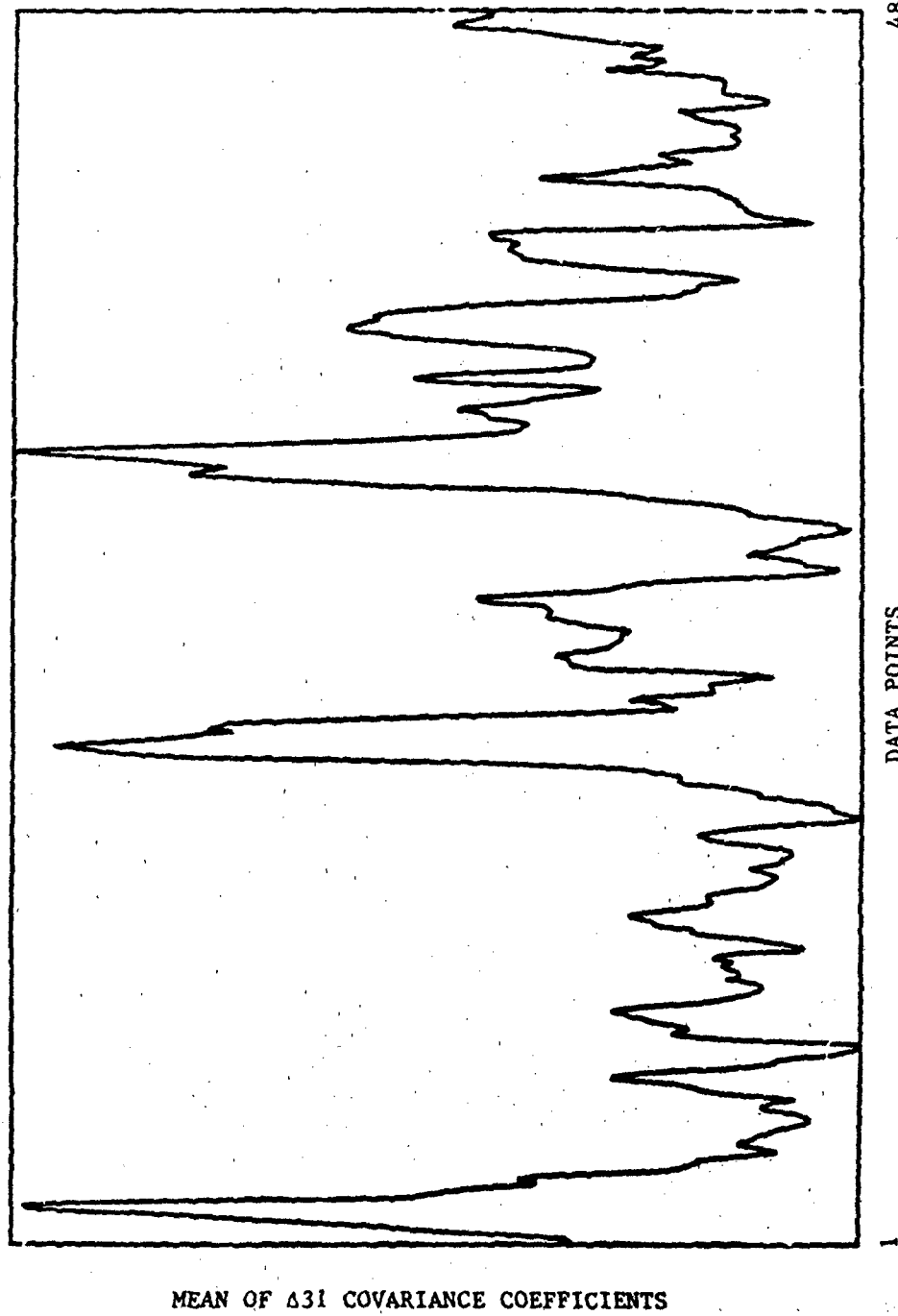


Figure 32. $\Delta 31$ covariance mean of $\Delta 11$ covariance means of individual rats during interval $T\phi$.

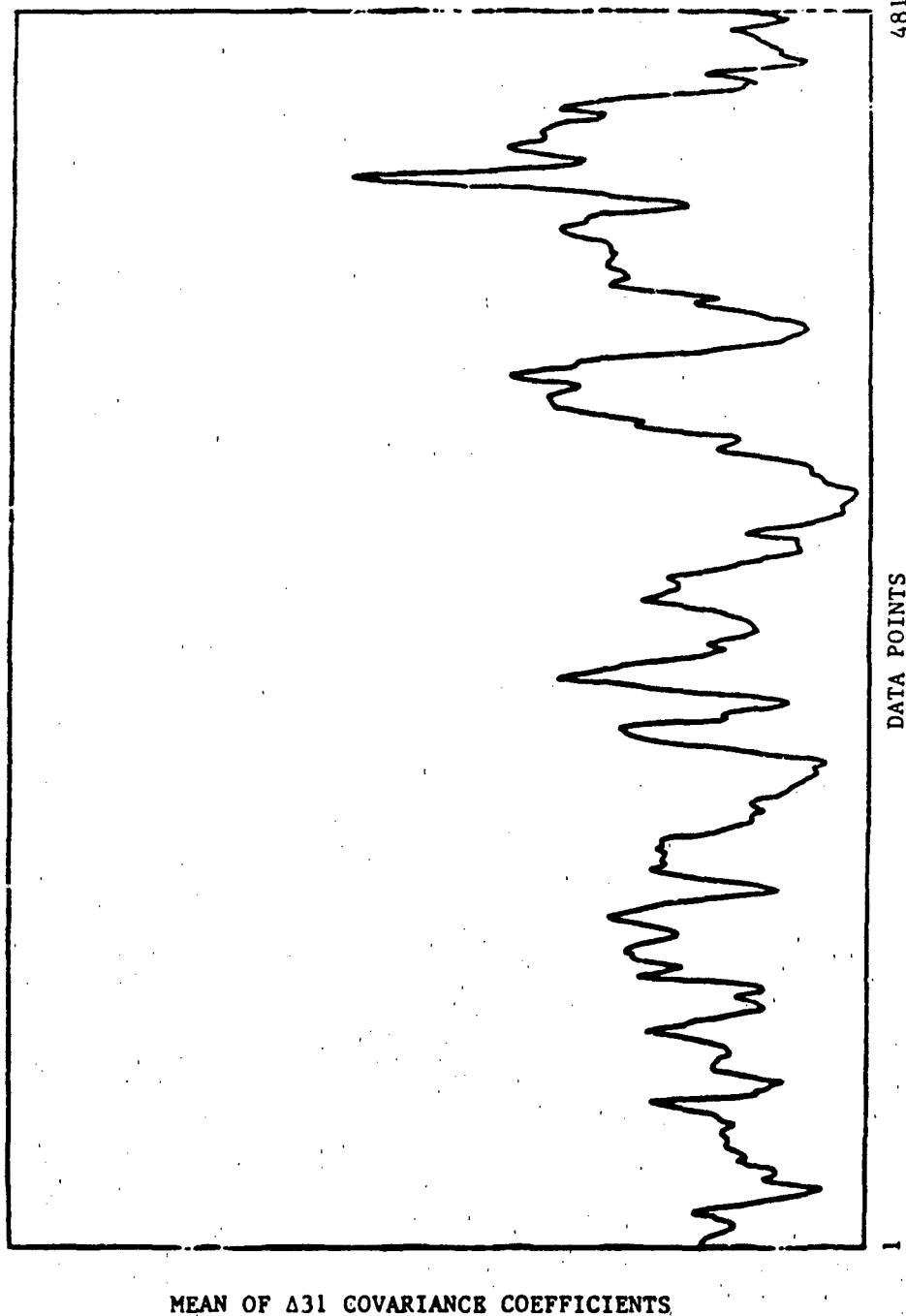


Figure 33. $\Delta 31$ covariance mean of $\Delta 11$ covariance means of individual rats during interval T+1.

MEAN OF $\Delta 11$ COVARIANCE COEFFICIENTS

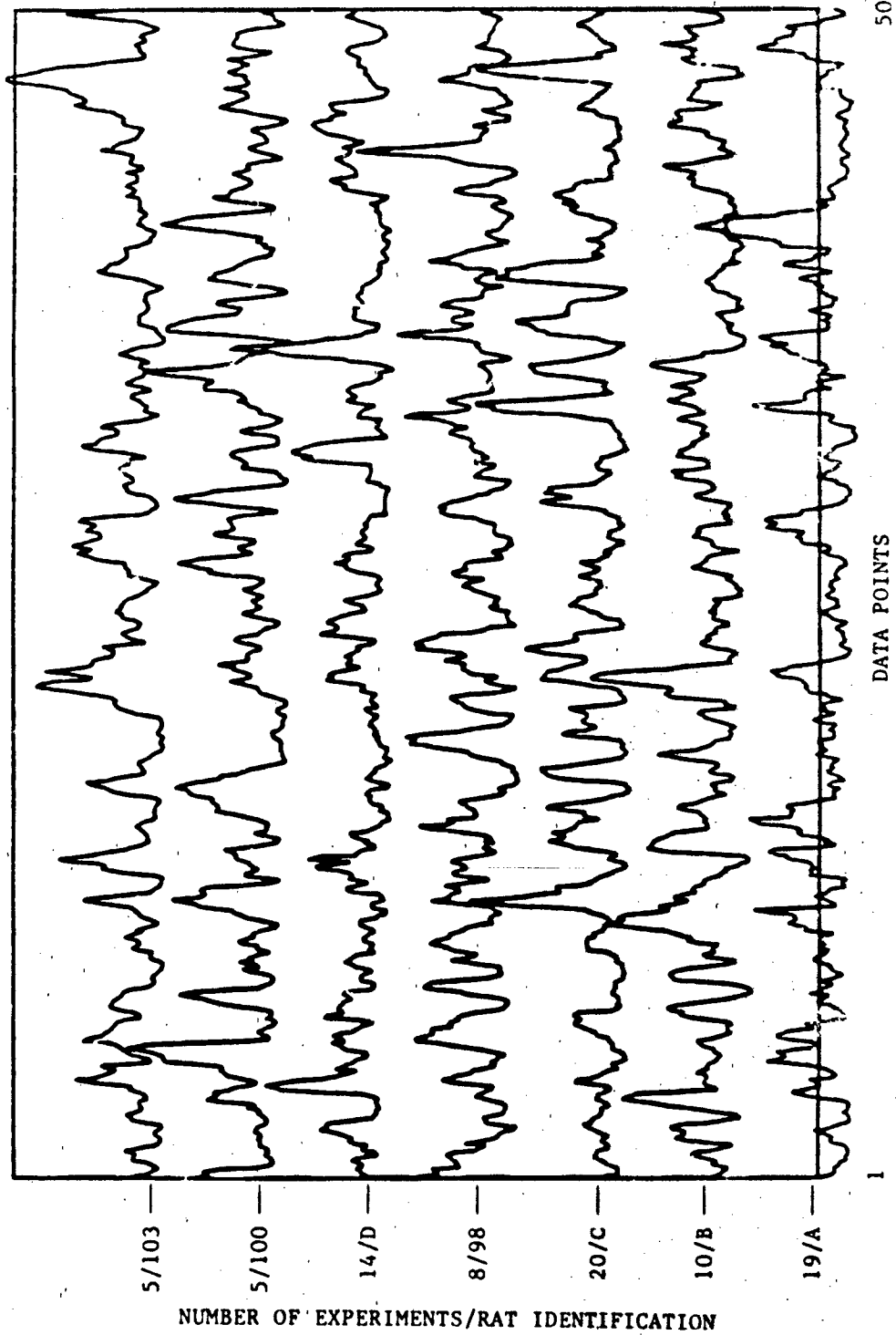


Figure 34. Normalized $\Delta 11$ covariance means by rat during interval N-1.

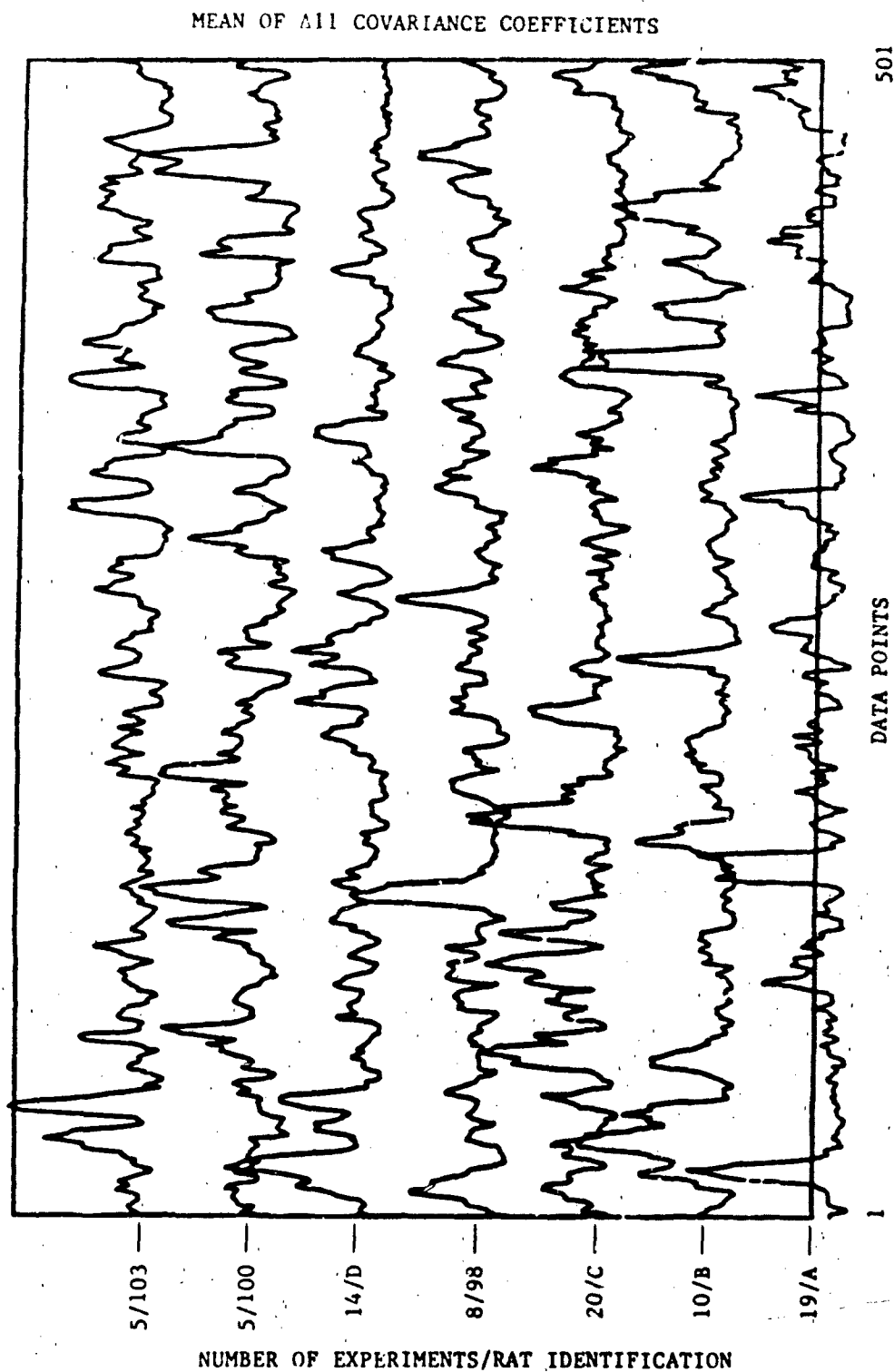


Figure 35. Normalized $\Delta 11$ covariance means by rat during interval Δt .

MEAN OF ALL COVARIANCE COEFFICIENTS

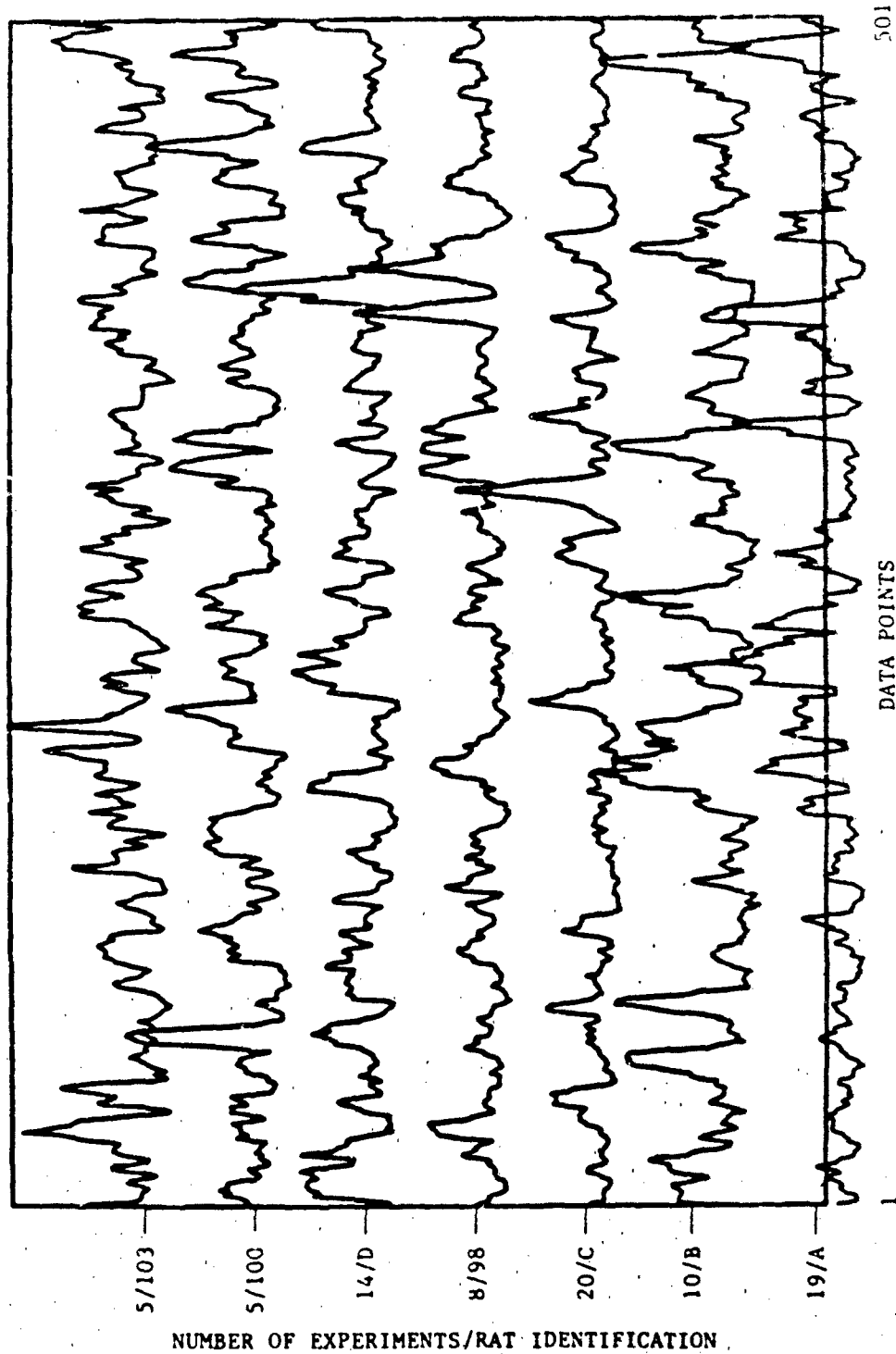
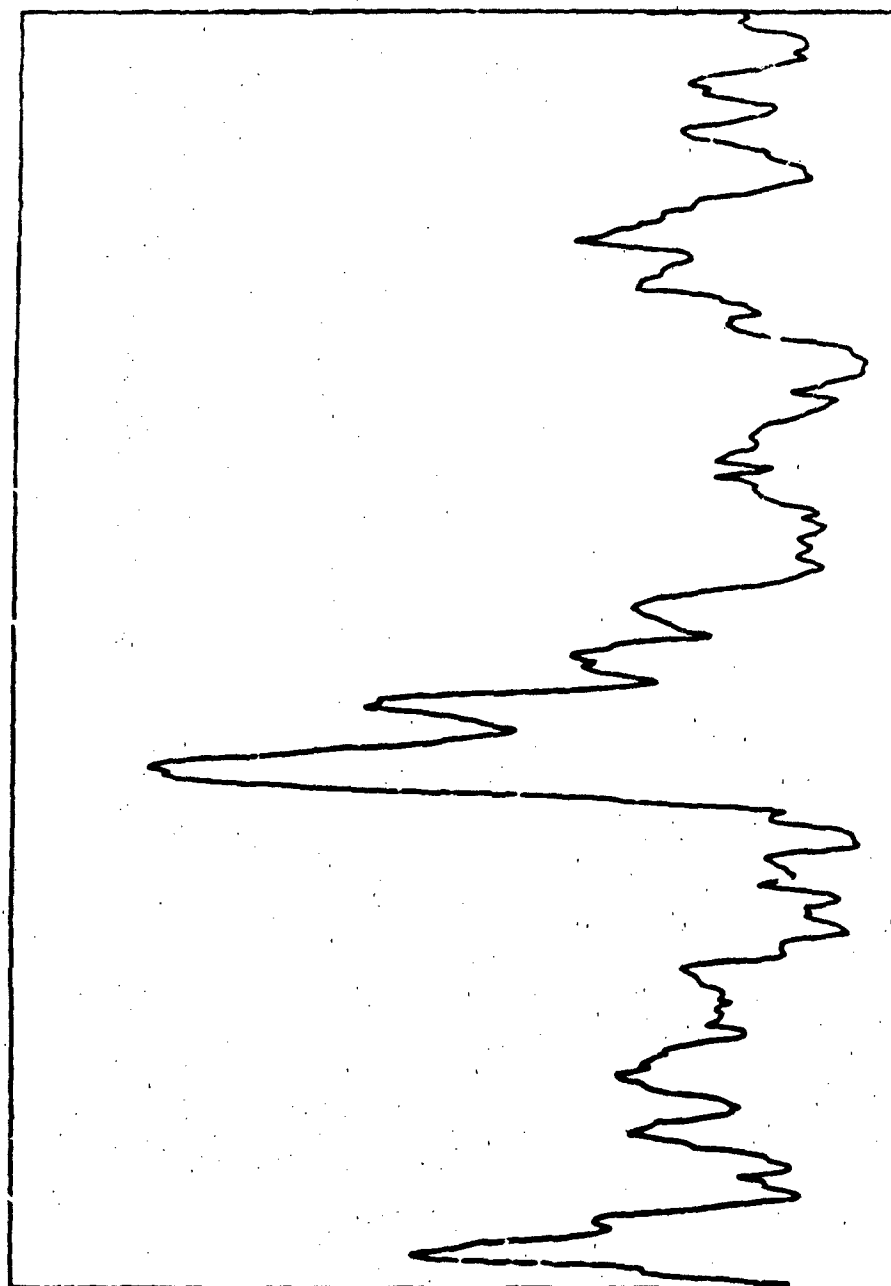


Figure 36. Normalized $\Delta 11$ covariance means by rat during interval N+1.

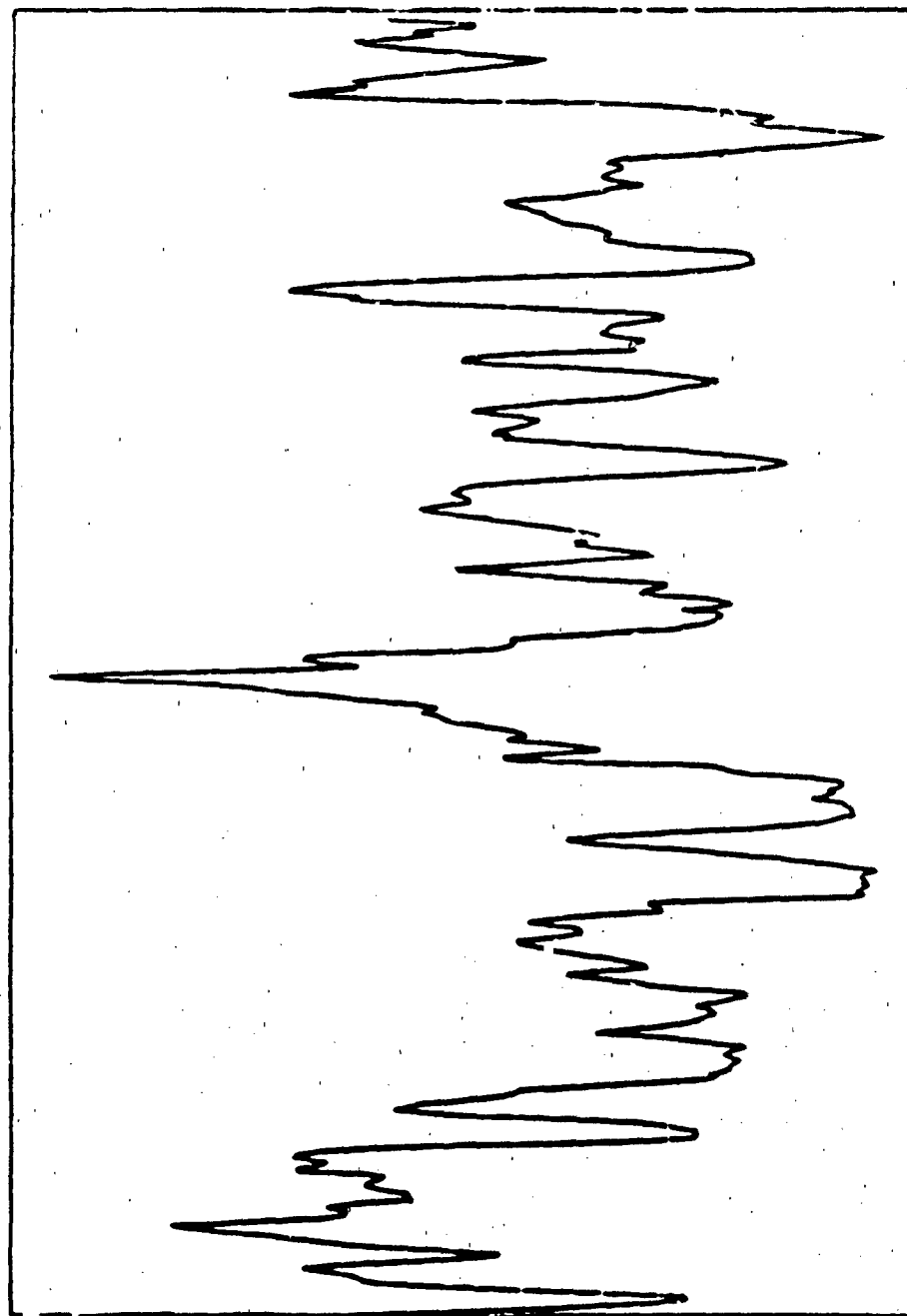


DATA POINTS

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MEAN OF $\Delta 31$ COVARIANCE COEFFICIENTS

Figure 37. $\Delta 31$ covariance mean of $\Delta 11$ covariance means of individual rats during interval N-1.



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DATA POINTS

Figure 38. $\Delta 31$ covariance mean of $\Delta 11$ covariance means of individual rats during interval No.

MEAN OF $\Delta 31$ COVARIANCE COEFFICIENTS

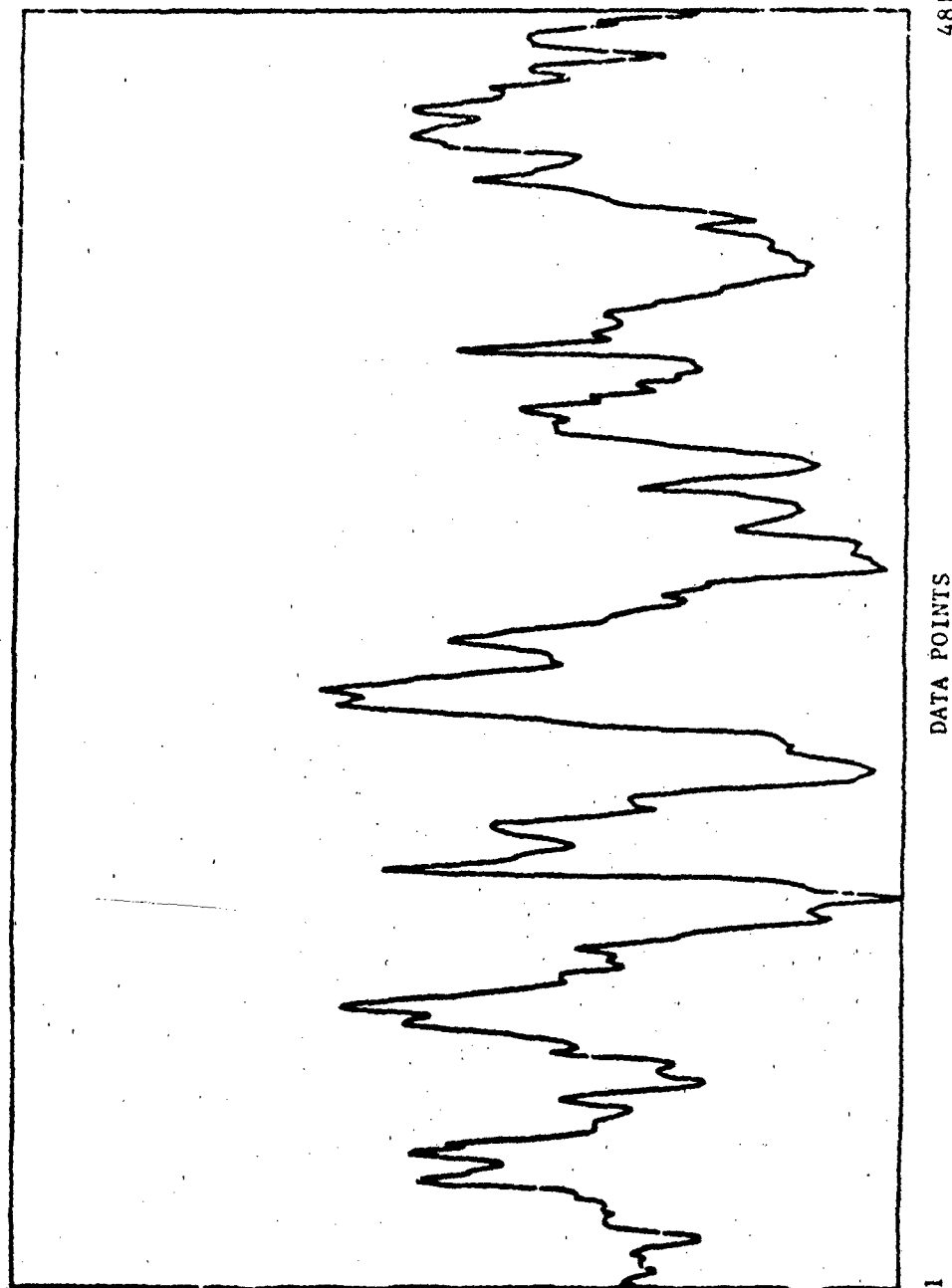


Figure 39. $\Delta 31$ covariance mean of $\Delta 11$ covariance means of individual rats during interval N+1.

In order to maximize the covariance of the desired signal features, one may vary the width of the sampling window, Δ , until the optimum mean value of covariance coefficients is obtained for the specific feature desired. It must be kept in mind during this process that the number of covariant events will decrease as Δ is increased, and conversely, more events will covary as Δ becomes smaller.

This relationship is evident from consideration of the nature of random and pseudo-random events. Given a window which is narrow and which examines a large ensemble of events, the probability of two or more events carrying in the window is much greater than for the case of a wide window which examines a much larger population of the same data.

For the data used in the proof of Thesis D (obtained from Experiment II) the computer was used to plot covariance means versus window width for all data point values of Δ between 1 and 100 for a specific feature, such as that seen at data point 220 in Figure 25. For this and certain other plots, the optimum value for Δ was found to be 11 data points, and this is designated on all appropriate plots as Δ_{11} . Six of the Figures (Figures 31, 32, 33, 37, 38 and 39) were plotted using $\Delta = 31$ data points. The reasons for the different window sizes will be discussed in the text to follow.

d. Covariance in EEG Signals. It is important to consider the "physical" significance of the covariance process as it relates to the data of the experiment. The EEG is not a satisfactory stationary event taken as a whole, since the signals which comprise this waveform are apparently the result of neural events which are individually non-cyclic, or at best aperiodic, for the most part. There are certain muscle artifacts which are cyclic and even somewhat constant in frequency over short time periods (breathing, heart action, etc.) but there is no sound evidence that there is significant cortical activity related to these routine events. Occasional intense muscle activity, such as heavy, deep sniffing, clawing, gnawing, and so on, may cause motor cortex activity which is briefly a stationary event in the EEG, and these cortical activities can conceivably occur in time synchrony with other events, such as the solenoid activation signal (the trigger signal). Further, it is extremely likely that muscular activity such as this can cause EEG artifacts due to muscle action potentials, especially if these potentials originate in the general area of the head and neck.

Since the covariance process serves to define areas of signal features across an ensemble of data which vary in the same manner but which do not necessarily exhibit similar degrees of amplitude variation, it is always possible that a large motor signal could by chance tend to covary as does a cortical neural signal of much lower amplitude which was generated in response to olfactory stimulus recognition and EBS anticipation, thus causing greater degrees of covariance than would exist for an AESC alone. It is not possible, a posteriori, to identify muscle action potential artifacts, nor was it feasible to

continuously observe and record muscle activity in the test subjects. The rats were, as described earlier, radically restrained and did not appear to tend toward either continuous or even sporadic struggling after accommodating to the restraint, but head and neck musculature was most certainly in action during some portion of all test periods, and thus one must cite these structures as the probable source of much of the EEG artifact content.

e. Detail Consideration of the Signal Analysis. Prior to discussion of the specific details of covariance signal analysis and feature extraction, it is necessary to define the various terms used in the signal processing output plots.

As noted in an earlier section, the basic data epochs in the digital data consisted of the time intervals ranging from 4 s prior to the trigger to 4 s subsequent to the trigger. These epochs, in turn, were extracted from the continuous analog data which contained several hours of background signals interspersed with the test periods in which 10 s of olfactory stimulus was applied. As preliminary analysis progressed, it became obvious that the 8-s digital data epochs were much too long, and even the slowest "learning" signal processor would need only 1 s of pre-stimulus data to enable it to evaluate the update signal data from periodic search intervals which would consist of olfactory samples of perhaps 2 to 3 s duration.

Consequently, the final data epochs were reduced to three 1-s intervals defined as follows:

(1) Interval $T - 1$: The period of data occurring in the 1-s interval immediately prior to the trigger.

(2) Interval $T\phi$: The period of data occurring in the 1-s interval immediately subsequent to the trigger.

(3) Interval $T + 1$: The period of data occurring in the 1-s interval immediately subsequent to the $T\phi$ interval.

(4) The trigger, as noted earlier, is defined as the leading edge of the d.c. pulse applied as the activation signal to the odorant-delivery solenoid valve relays.

(5) $\Delta 11$ is the symbol used to designate those covariance processes in which the window width was 11 data points.

(6) $\Delta 31$ is the symbol used to designate those covariance processes in which the window width was 31 data points.

(7) The mean of the covariance coefficients, seen as the abscissa on most plots, is specifically defined in equation 6.

With these terms in mind, it will now be of interest to examine Figures 25 through 39. Figure 40 depicts a small segment of the raw analog monopolar signals obtained from the cingulate electrode of Rat C. Here are preserved 25 epochs of the interval T_0 with the relative amplitudes of the signals maintained in true relationship to the original signals.

In this figure there appears to be little similarity among these epochs even after considerable perusal by the naked eye, but this is not surprising since this research would have been completed some years previously had such simple evidence of an olfactory-induced AESC been available.⁸⁹ Data of the general form of that in Figure 40 were tested against each of the mathematical processes described earlier in this section, and, as noted, little of consequence to the proof of Thesis D was observed. Almost from its inception, however, the covariance process began to elucidate signal features which were quite significant, since they were not only highly visible, but, most importantly, they were consistently positioned in time such that their occurrence coincided with the time of known stimulus-related events, such as trigger origin and odorant bolus arrival.

Figure 25 depicts an early result of the application of the covariance process. In this plot one observes two outstanding events. First, there is a large peak near the left edge of the plot. Since the abscissa of all figures (in the group of Figures 25 through 39, excluding Figure 30) represents 1 s of time—minus the window width—and since the data sampling rate is 512 data points/s, the first large peak seen in Figure 25 occurred at about 40 ms subsequent to the activation of the TNT odorant solenoid valve relay. At first, this event was assumed to be perhaps fortuitous, but after examination of 20 or more other, similar data epochs across the rat population, it became apparent that this peak must be related to the solenoid valve relay activation in a relatively invariant manner. Such a repetitive event was seen to exist also in all plots of covariance coefficient mean values where the neutral stimulus odorant-delivery solenoid activation pulses were the genesis of the trigger. The most reasonable explanation of this ubiquitous signal feature is that it represents the effect of the relay "click" on the auditory processing circuits in the rat brain!

⁸⁹ Abundant data of this nature were available for monopolar signals obtained from the parietal and occipital electrodes and from the bipolar combinations of these electrodes (parietal-occipital, parietal-cingulate, and cingulate-occipital) but trial analyses led me to conclude that the optimum AESC features were to be found in the cingulate electrode data. The bipolar data were not examined in great detail since these were opportunistic data taken because it was a simple matter to achieve, and since these data could offer nothing which was unavailable in the monopolar data. The remaining monopolar data from the parietal and occipital electrodes could be examined in greater depth at some future date, but for this research, little could be gained from the additional effort expended in such analysis.

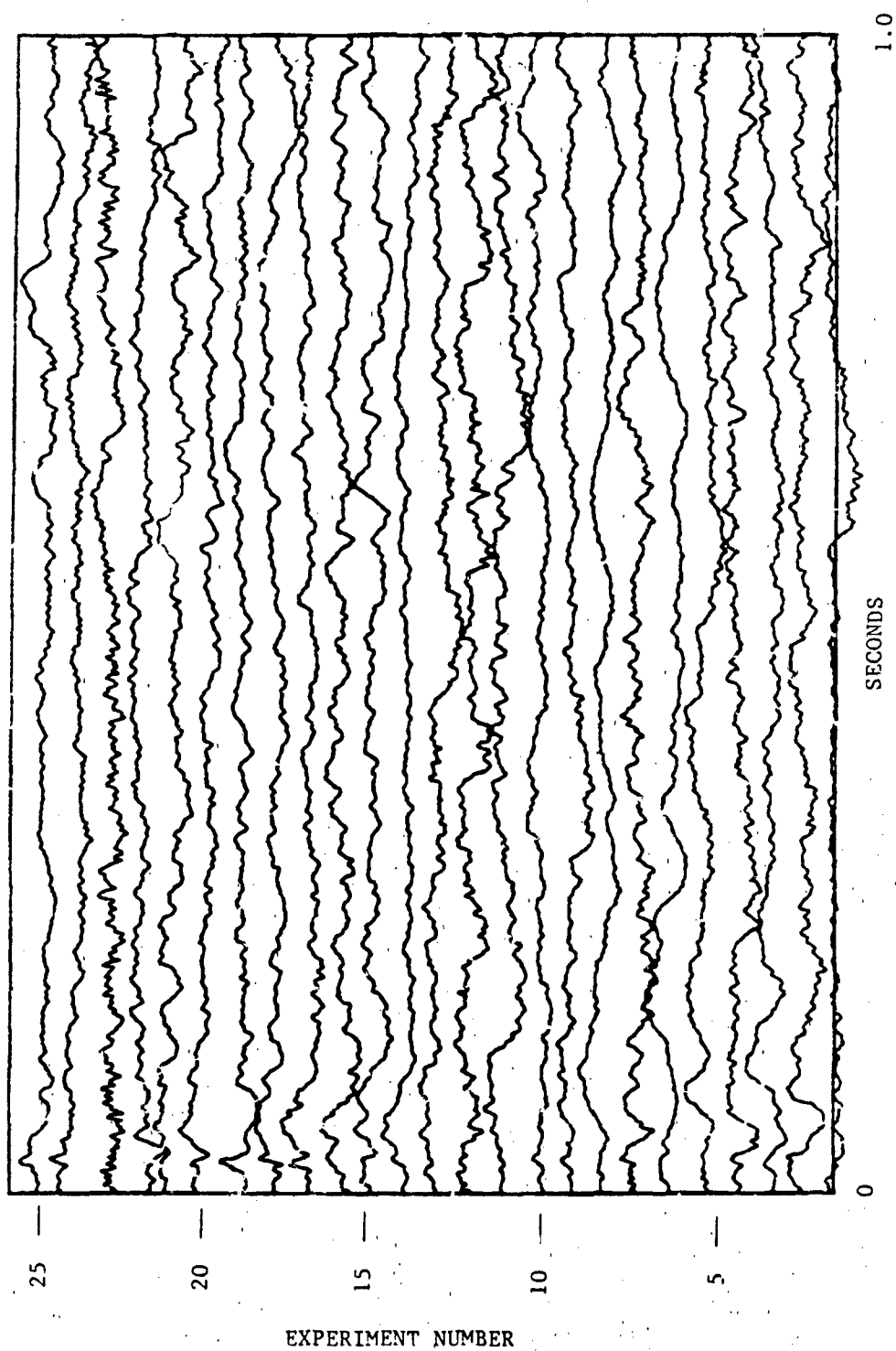


Figure 40. Twenty-five monopolar raw data epochs for interval $T\phi$ for Rat C.

It must again be emphasized that, from the beginning, there was intention to attempt to explain the physiological processes involved in this research. The protocol sought only to prove the existence of a unique event in the EEG subsequent to exposure to the TNT stimulus event. This philosophy remains operational and the only reason for advancing a supposition as to the nature of the peak at 0 ms lies in the unique value of this signal feature to the analysis process as a time mark and to its presence across all epochs for all olfactory stimuli.

While published data as to the value of the auditory physiological processing lag, or latency, are scarce, it appears that the observed 40-ms latency in the test subjects is comparable to that observed in humans. Since the relays were physically located at about 1.5 m from the Faraday Box and since the click can be clearly heard on the voice channel of the analog data tapes, one must assume that most if not all rats could hear the solenoid activations. Considering that the activation click originated within 2 ms of the application of the trigger (the beginning of the 1-s data period of Figures 25, 26, 28, 32, 35, and 38) and considering that the sound transit time was about 3 or 4 ms, a physiological latency of about 30 ms added to these delays would cause an auditory signal feature to exist within the limits observed in the data. Thus this signal feature has been termed the "auditory event" and will be so designated in the test to follow.⁹⁰

The plot data for Figure 25 were derived from the raw digital data by the covariance process by using a window, or Δ , of 11 data points. Note that this figure is actually an enlarged plot of the cascade trace labeled 25C in Figure 28. The auditory event is clearly evident as it is routinely in $T\phi$ and $N\phi$ interval plots. Figure 25 shows evidence of olfactory stimulus recognition, but, standing alone, this evidence would not suffice for verification of Thesis D.

Figure 26 shows the $\Delta 11$ covariance means across the entire ensemble of 165 experiments of neutral and TNT stimulus events. Figure 27 displays the plots of $84 T - 1$ experiments in which TNT was to be the olfactory stimulus; Figure 28 is the plot of the $T\phi$ interval of this data set; and Figure 29 is the plot of covariance means for the interval $T + 1$. In these plots the ordinate represents the value of the mean of the unique covariance matrix coefficients (excluding the diagonal and the "image" half-matrix). Since the window is 11 points, the total span of the abscissa is seen to represent $512 - 11$, or 501 data points.

⁹⁰ From these considerations and from detailed study of the many data plots obtained from earlier covariance efforts, certain basic operating parameters of a potential rat-biosensor explosives detector became apparent. It was fortunate that the auditory signal from the odorant-delivery solenoids was available to the rats since this signal could serve as an alerting signal which "supersensitizes" the detector rat to the imminent presence of an odorant, and, of course, the auditory peak in the EEG could serve as a monitor of the state of awareness of the detector rat. Future microprocessors programmed to recognize the covariance-derived signal features to be described below can employ the auditory peak as a "go-ahead" signal.

Figures 27 and 29 will be discussed in conjunction with Figures 31 and 33. Of special interest here is Figure 28. Several significant signal features are evident in Figure 28. The most evident signal is the auditory event seen at about data point 20, which is near the left edge of the plot. No similar structure is evident in either the plots of $T - 1$ or those of $T + 1$, and this observation further accentuates the legitimacy of the assumption that the $T\phi$ plot event at 40 ms is indeed of auditory origin.

Since the transit time of the odorant bolus is known to be 500 ± 70 ms, a careful examination of the mid-abscissa region of the $T\phi$ plot in Figure 28 may reveal some evidence of unusual covariance at about data point 220; but in these cascade ensemble plots, the feature is not as immediately apparent as one might wish. However, after some study, it became apparent that these unique signal covariances at or near data point 220 occur only on the plots for $T\phi$.

In order to make the feature at data point 220 more evident, a second application of the covariance process was effected on all $\Delta 11$ data sets. The results of this process are shown in Figures 31, 32, 33, 37, 38, and 39. Prior to effecting this processing, it was again necessary to optimize the window width for the set of features known to exist near data point 220. Figure 30 displays the process whereby a trial plot similar to Figure 32 was analyzed for optimum Δ . The trial plot was the $\Delta 11$ covariance of the sum of the plots of Figure 28. While the trial figure resembled Figure 32, $\Delta 11$ was not optimum, as seen from Figure 30 where the peak occurs at $\Delta 23$ with essentially a flat-top response to $\Delta 31$. As noted earlier, a larger window is a better choice than a narrow window, since only events with similar covariance characteristics will tend to peak in a large window, and thus the most accurate feature identification is obtained. $\Delta 31$ was then selected and Figures 31, 32, and 33 were generated. These plots, then, represented the $\Delta 31$ covariance mean of the $\Delta 11$ covariance means observed in the plots of Figures 27, 28, and 29.

Figure 32 is of great significance to this research effort since it represents graphical evidence of the validity of Thesis D!

To recapitulate the events displayed in Figure 32:

- (1) 84 separate experiments derived from the data for Rats A, B, C, D, 98, 100, and 103 were analyzed over the $T\phi$ interval.
- (2) $\Delta 11$ covariance means were obtained for the ensemble of 84 experiments.
- (3) $\Delta 31$ covariance means were derived for the summed results of the $\Delta 11$ calculations.
- (4) The unique features seen in Figure 32 were obtained only for TNT stimuli.

It is pertinent now to move to Figure 38 which is the equivalent to Figure 32 except that in this figure the data for neutral stimuli in the $N\phi$ interval were examined. One may observe the clearly evident auditory signal at about 40 ms—data point 22 is the median point—into the $N\phi$ interval. However, there is no significant covariance observed at data point 220, and there is no significant pairing such as that seen in Figure 32. *The special significance of the initial peak in Figure 32 at data point 220 is that this represents the earliest arrival time of the odorant bolus (about 430 or 440 ms post-trigger).* Likewise, the singular configuration and amplitude of the departure of these peaks (for the short period of 150 ms or thereabouts) from the background covariance when the TNT odorant first arrives, is also significant.

In Figure 38, the earliest $N\phi$ peak which could possibly be related to an olfactory stimulus occurs at about data point 290, which represents a time just beyond the upper measured limit of odorant arrival time. Thus, while the single large peak in the center of $N\phi$ ensemble may or may not represent some olfactory related event, there is little doubt that the twin peaks seen in Figure 32 are evidence of an event related only to the presence of TNT vapor. There is, of course, no valid manner in which one might verify that this event is truly anticipatory, but judging by the behavioral observations made over hundreds of experiments, the test subjects certainly appear to experience anticipation at the first arrival of TNT vapor. From this subjective evidence, it was determined that this event will continue to be called an Anticipatory Evoked Spectral Change in the EEG.

Since all data taking sessions resulted in interlaced TNT and neutral olfactory stimulation in a pseudo-random manner, there can be no reason to assume that the data are time or event biased in any manner which might accidentally prejudice the results.

Returning to the remaining Figures 27, 29, 31 and 33 which are in the TNT set, it will be seen that Figures 27 and 31 representing the $T - 1$ interval, and Figures 29 and 33, representing the $T + 1$ interval, show no features as significant as the outstanding twin covariance peaks of Figure 32. One would not expect to find any features related to the stimulus events in the $T - 1$ interval of Figures 27 and 31 since the test subject has been in an unstimulated state for random periods ranging in length from 20 s or 30 s to perhaps 60 min or more prior to the $T - 1$ interval, and thus the EEG should show only the homeostatic processes typical of the restrained animal. Intervals $T\phi$ and $T + 1$ obviously also contain features resulting from the homeostatic processes, and interval $T + 1$, shown in Figure 33, was presented primarily to demonstrate that the TNT recognition feature elicited by the covariance process appeared to exist only in the $T\phi$ interval.

Finally, the Figures 34, 35, 36, 37, and 39 must be examined. Figures 34, 35, and 36 are the neutral stimuli homologues of the TNT stimulus plots of Figures 27, 28, and 29, and Figures 37 and 39 are the homologues of Figures 31 and 33. Figure 34 is the plot of the covariance means for the full second immediately prior to the trigger (the $N - 1$ interval), while Figure 35 is the plot of the 1-s interval immediately subsequent to the trigger (the $N\phi$ interval) and Figure 36 is the plot of the $N + 1$ interval, which is the 1-s interval following the $N\phi$ interval.

As was the case with the same time intervals for the TNT stimulus events, the $N - 1$ and the $N + 1$ interval plots show no features which have significance to this program. Figures 34, 36, 37, and 39 further show the quasi-stationary nature of the background EEG. Nothing of significance to olfactory processes is discernible in the $N - 1$ interval of Figures 34 and 37 for the reasons noted for the $T - 1$ interval. Likewise, it is apparent that no olfactory events are seen in the $N + 1$ interval.

Based upon the foregoing analysis, it is virtually certain that a unique feature appears in the output of the Covariance Process when—and only when—the input EEG is derived from a fully conditioned test subject during a period of olfactory stimulation by TNT vapor. Since the elucidation of a specific EEG feature thus derived was the research goal in the effort directed toward Thesis D, the Covariance Process could have served admirably as the single metric for the proof of Thesis D. However, the continual search for suitable signal processing techniques led to the development of a second process which was also capable of recognition of a unique EEG event and which appears not only to verify the results of the Covariance Process, but which also gives an insight into the nature of the Anticipatory Evoked Spectral Change. This process—the Segmentation Process—in concert with the Covariance Process, leaves no doubt as to the existence of the unique feature called for in Thesis D.

f. The Segmentation Process. The second mathematical technique which offered confirmation of Thesis D was devised by the BDM Corporation of McLean, Virginia, under the guidance of Dr. Igor Frolow. The basic metric is much akin to the Spectral Separator process described earlier. In the Spectral Separator process, the 1-s data interval prior to the trigger was compared to the 1-s data segment immediately subsequent to the trigger with a window width of 1 s, whereas the Segmentation Process examines the same intervals (and more) by scanning, using a small stepping window. In addition, the Segmentation Process offers an update function which places this scanning, iterative process into the category of an adaptive feature extraction technique. As a result of these additional steps, the Segmentation Process was capable of detecting the AESC when the less complex Spectral Separator could not.

The basic process is defined below:

- (1) Define an initial window W_0 consisting of the first N points of a given time series.
- (2) Describe the signal within the window using an appropriate measure such as the power spectrum.
- (3) Move along the time axis in increments of 1 points, each time defining a new window of length N and computing the corresponding measure.
- (4) As long as the new measure, when compared to that of W_0 , is less than a pre-specified level β , step 3 is repeated. Otherwise, identify the beginning of a new segment and return to step 1, using the remaining time series in all further calculations.

Stated differently, the process is defined as:

Let the raw EEG data be expressed by the Fourier transform

$$A_j(m) = \frac{1}{N} \sum_{t=1}^N x_j(t) \cos 2\pi \frac{mt}{N} \quad (18)$$

$$B_j(m) = \frac{1}{N} \sum_{t=1}^N x_j(t) \sin 2\pi \frac{mt}{N} \quad (19)$$

The power spectrum then is

$$P_j(m) = A_j^2(m) + B_j^2(m) \quad (20)$$

where $P_j(m)$ = m th component of the j th power spectrum.

Likewise

$P_0(m)$ = m th component of the 0 th power spectrum.

The variance of the j th interval is

$$\sigma_j^2 = \frac{1}{N} \sum_{t=1}^N [x_j(t) - \bar{X}_j]^2 \quad (21)$$

where \bar{X} = sample means
and the variance of the 0th interval is

$$\sigma_0^2 = \frac{1}{N} \sum_{t=1}^N [x_0(t) - \bar{X}_0]^2 \quad (22)$$

Let N be the number of data points in the window W_0 ; then the error function, or the degree of mismatch between the curve sampled in the 0th interval and all following (j)th intervals, is then defined as:

$$E_j = \sum_{m=1}^{N-1} \left[\frac{P_j(m)}{\sigma_j^2} - \frac{P_0(m)}{\sigma_0^2} \right]^2 \quad (23)$$

In physical terms, this technique may be envisioned by examining a segment of raw data such as that shown in Figure 16. If the analysis begins at exactly 1 s prior to the advent of the TNT trigger, the beginning of this interval will be the 0th position of the window, and this window will be termed W_0 . The window is then stepped across the segments of data in increments of 1 equal to 10 data points. At each step, W_1, W_2, \dots, W_j , the power spectral variation is equated against a predetermined threshold, β . If at any step the threshold is not exceeded, E_j is equated against the power spectral value determined in W_0 using the expression 6, above. If the threshold is exceeded, then the original W_0 is discarded and the value at W_j then becomes the new W_0 , which then remains fixed at its data point of origin until it, in turn, is replaced by a later W_j .

The selection of window width is of considerable significance since an injudicious choice can impair the Segmentation Process to the point of failure. The window size, as in the Covariance Process, can be varied between 1 and 512 data points. Realistically, the practical limits range between 32 and 256 data points since windows smaller than 32 will show only the highest frequency components, while windows larger than 256 data points may exceed the brief periods of quasi-stationarity which have been seen to exist in these data. After several trial processes, 128 data points were chosen to be the optimum window for the data of this research.

The threshold value of E_j , β , was selected by trial for this initial process. (Later versions of the Segmentation Process may allow for automatic threshold set.) The minimum value for β was found to be 0.150 for the data examined in this effort. To be certain that this threshold level was not too low, a detailed computation of β was made for the case of random noise. It was determined by this calculation that for truly random or "white" noise, the mean value of $E_j = 0.0155$ with $\sigma^2 = 0.0000095$.

Figure 41 depicts the results of application of the Segmentation Process to a single TNT stimulus experiment from the data set for Rat B. The window width was set—as it was throughout the final iteration of this process—at 128 data points. The value of β was 1.0. Note that the abscissa is the time axis, and, in terms of the Covariance Process terminology, intervals $T - 1$, $T\phi$ and $T + 1$ are contiguous in presentation in this and all similar figures in the set extending from Figure 41 to Figure 58. Since the experimental apparatus was known to manifest a 500 ± 70 ms delay between the trigger—which occurs in this figure at 1.0 on the Time axis—and the arrival of the odorant bolus, those events which occur at or near 1.5 on the Time axis are worthy of close scrutiny. In Figure 41, there is an event, beginning at 1.5 s which achieves an E_j or error function value of about 0.180. To investigate this region further, the process was rerun using $\beta = 0.175$.

Figure 42 shows the results of this step in the process. The first window, W_0 , in this and all figures, is positioned in time at the first significant departure of E_j from zero, which occurs in this figure at about 0.25 s. As W_j stepped across time, all power spectra were compared to the power spectrum of this W_0 and the values of E_j were plotted. The appearance of Figures 41 and 42 are thus identical (even though β is assigned different values) until the 1.5-s event is reached. In Figure 42, W_0 is then reset since the peak value of E_j now exceeds the threshold, and the W_j at this point now becomes the new W_0 . The character of the plot then changes. At time 1.7 s or thereabouts, this W_0 is replaced by the value of W_j at 1.7 s and an updated W_0 is fixed at this point and remains the comparison standard until 2.5 s, where the iteration again occurs.

In the search for the Anticipatory Evoked Spectral Change, the Segmentation Process uniquely satisfies the need to identify the nature of the spectral change. Figure 45 shows the spectral density function obtained when the first W_0 is positioned at 0.25 s. The value of the dominant frequency of the spectral density is seen to be about 4 Hz, which is extremely interesting since this dominance is known to occur in the so-called Theta band in mammalian EEGs when the animal is at rest (not at maximal alertness, but also not asleep). At time 0.25 those animals which had adapted to the test fixture had ceased to struggle against the restraint and were, insofar as visual observation could discern, at rest.

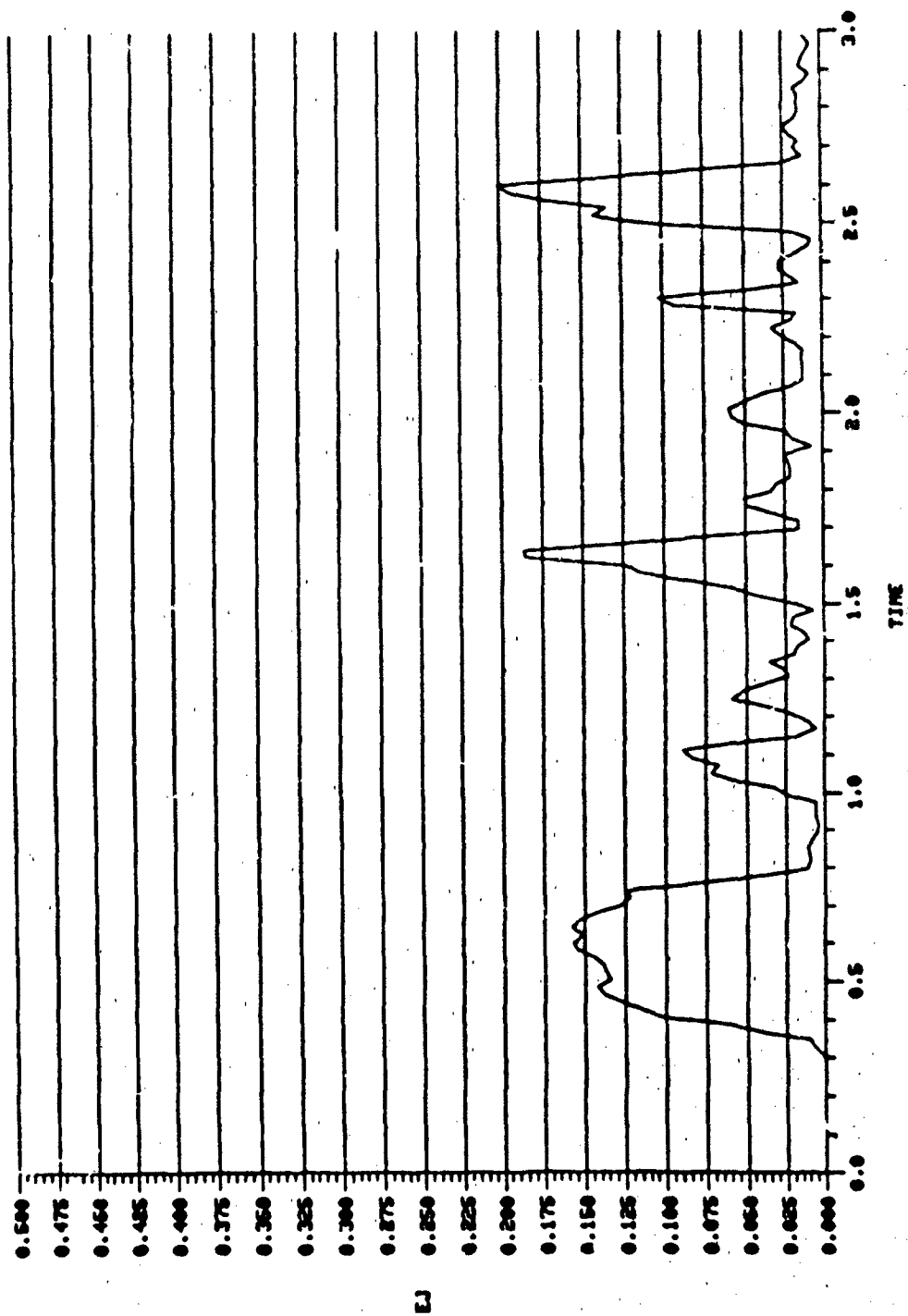


Figure 41. Error function versus time in data epoch for $\beta = 1.0$, TNT stimulus.

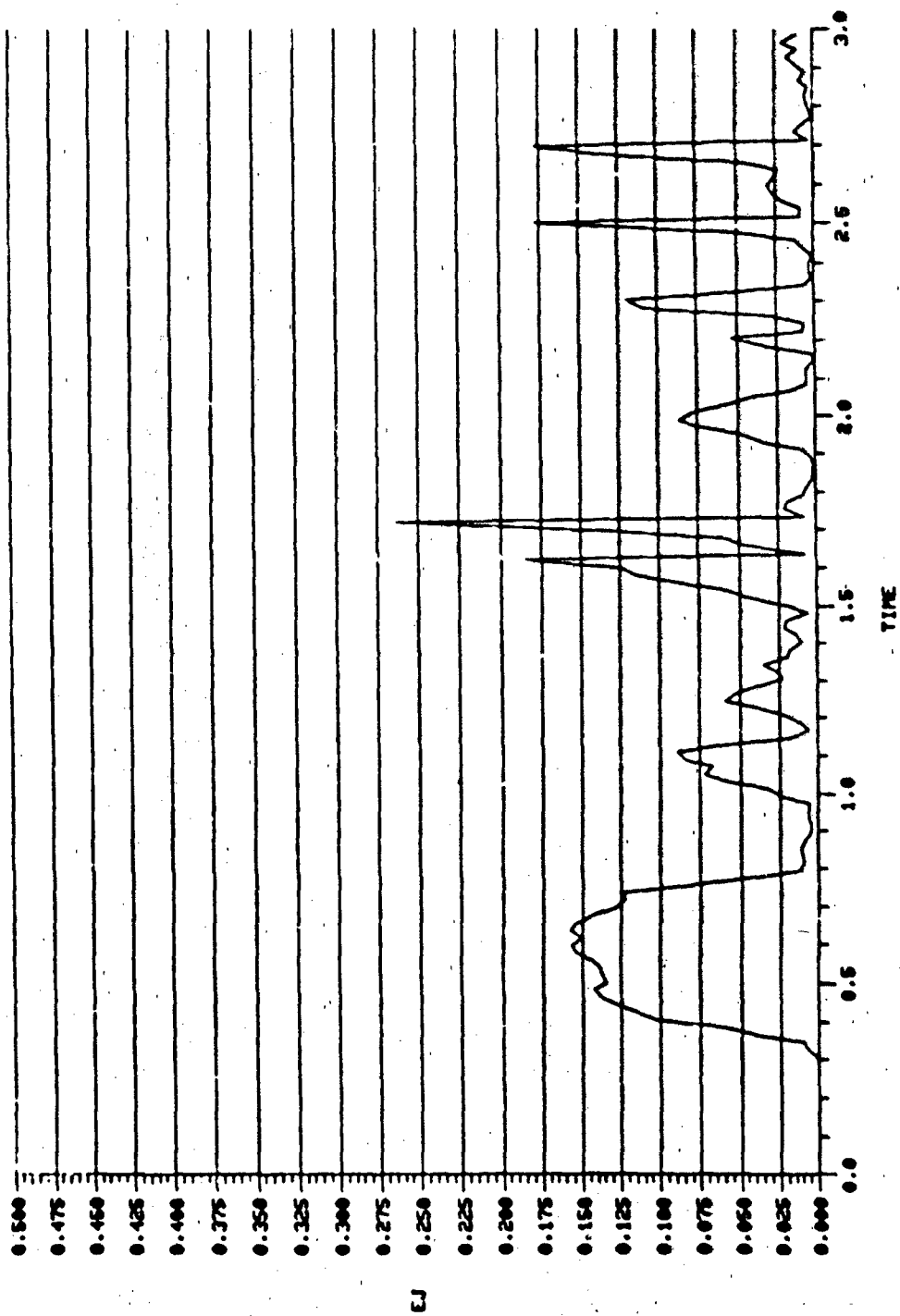


Figure 42. Error function versus time in data epoch for $\beta = 0.175$, TNT stimulus.

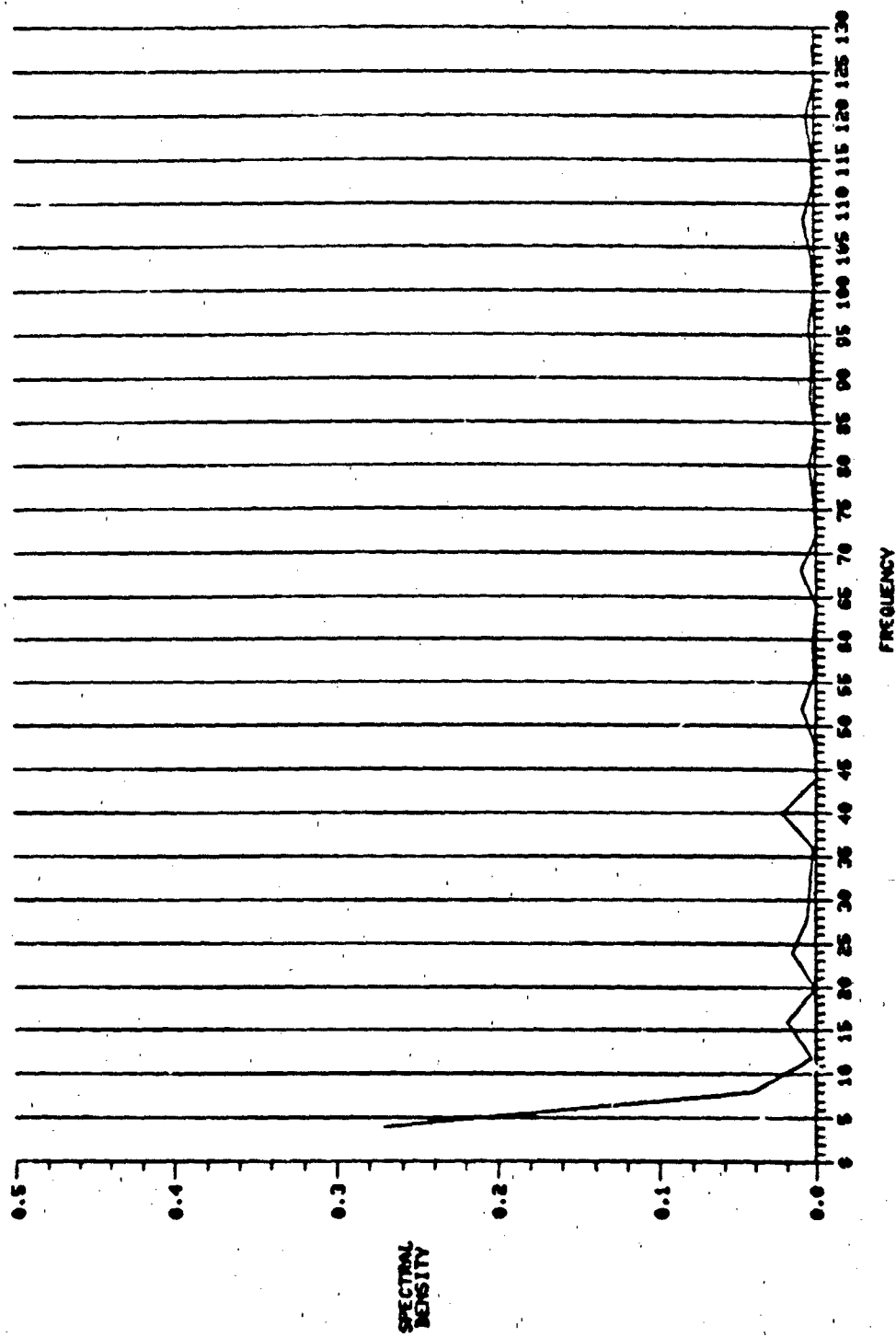


Figure 43. Spectral density function at first window of Figure 42.

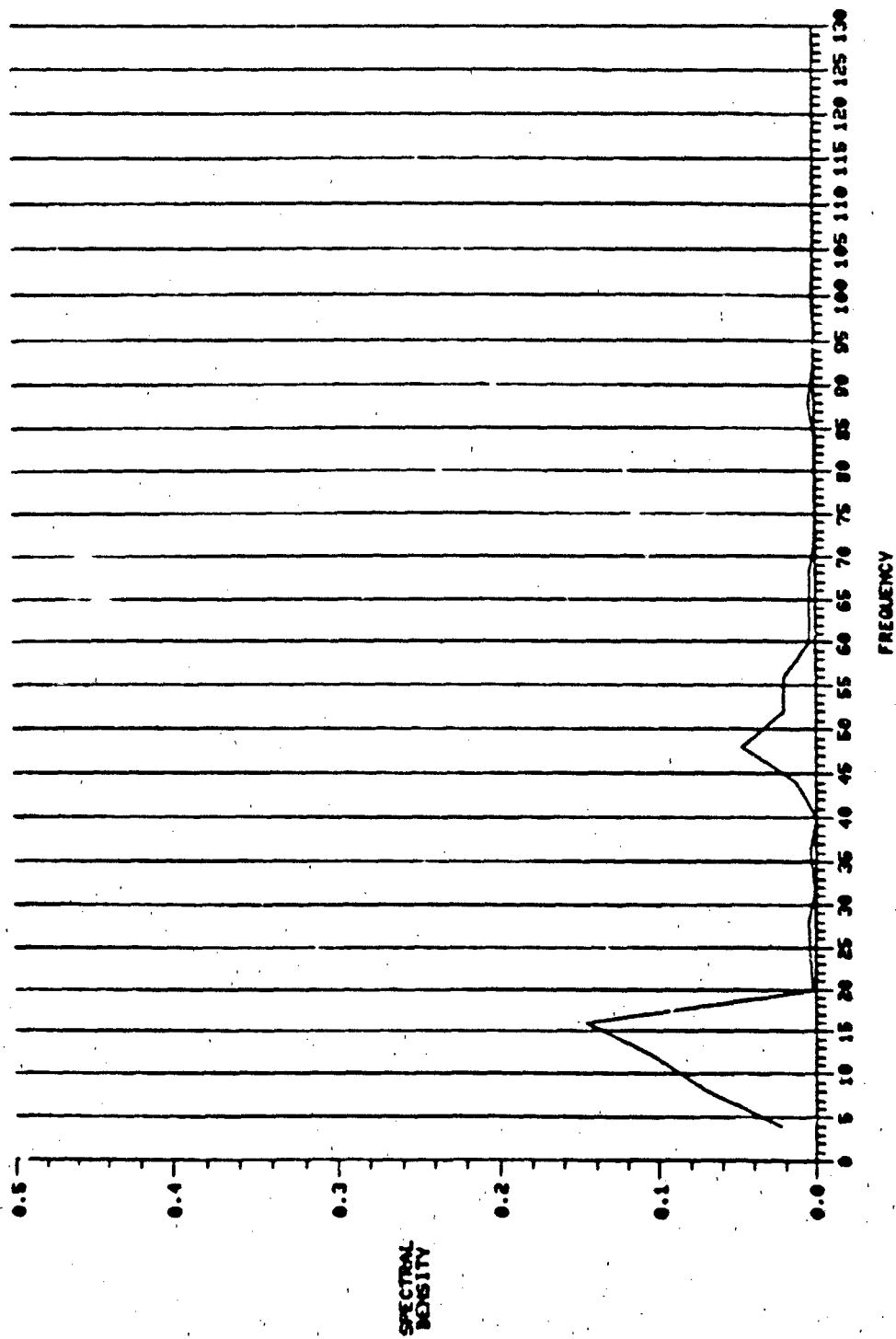


Figure 44. Spectral density function at second window of Figure 42.

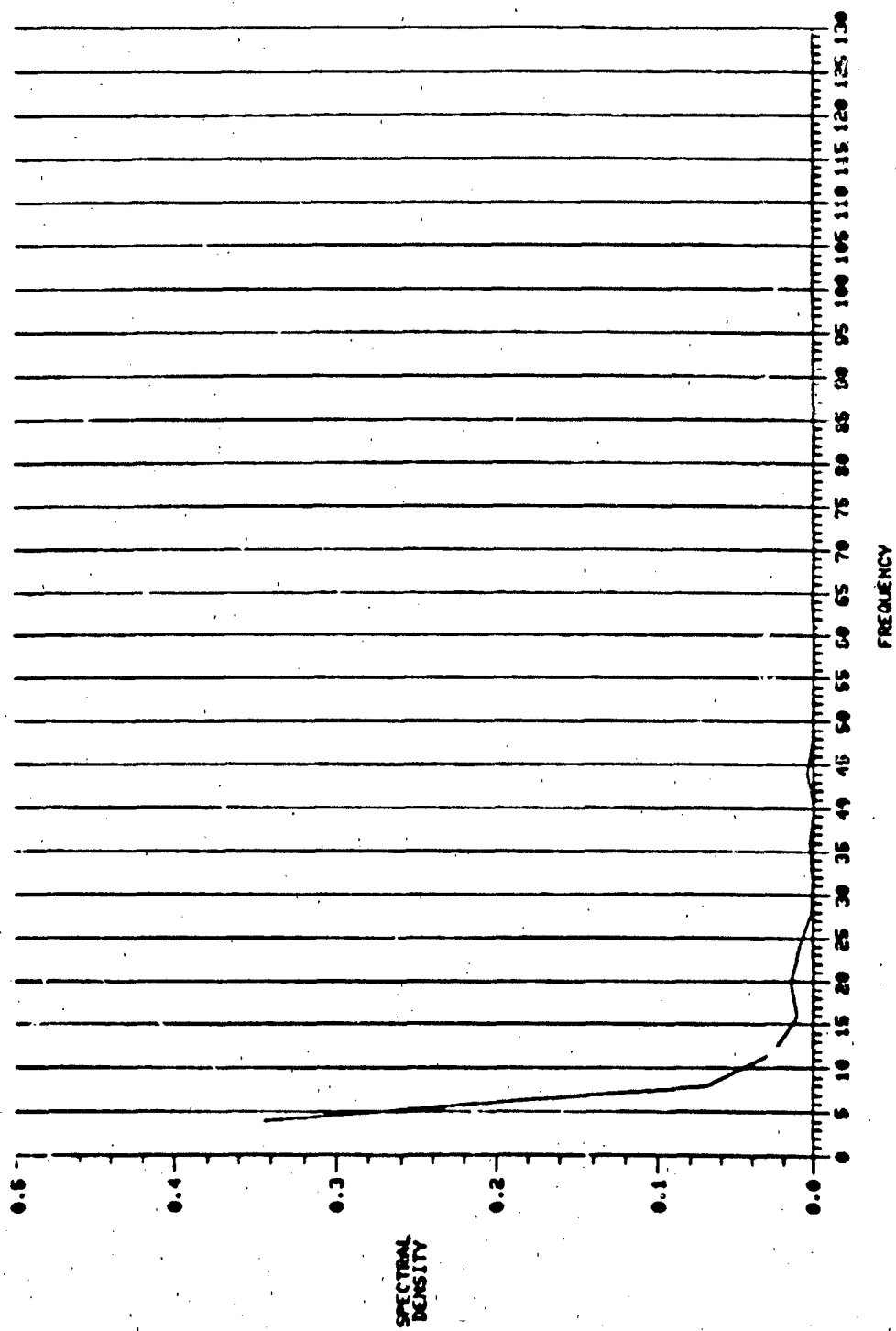


Figure 45. Spectral density function at third window of Figure 42.

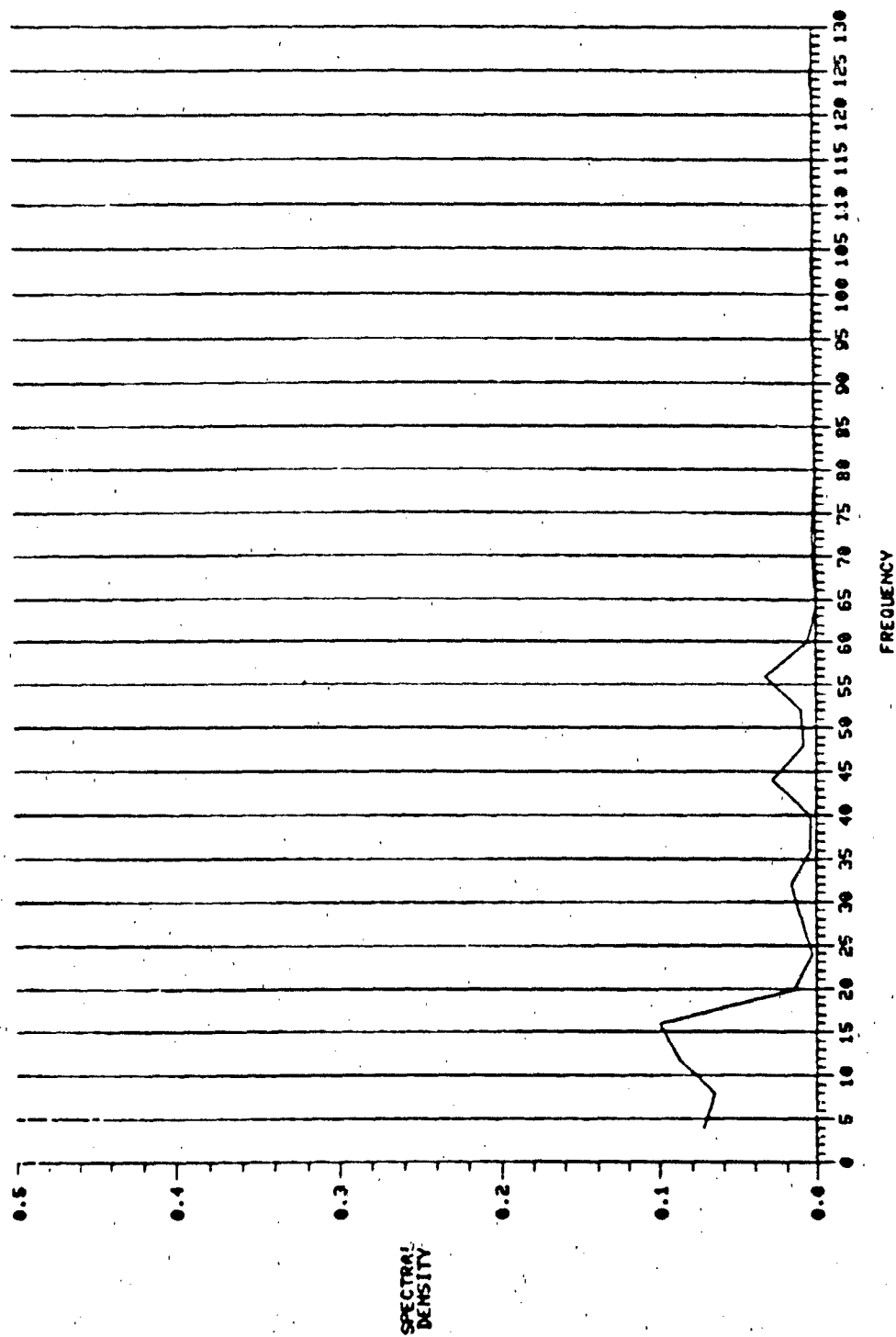


Figure 46. Spectral density function of fourth window of Figure 42.

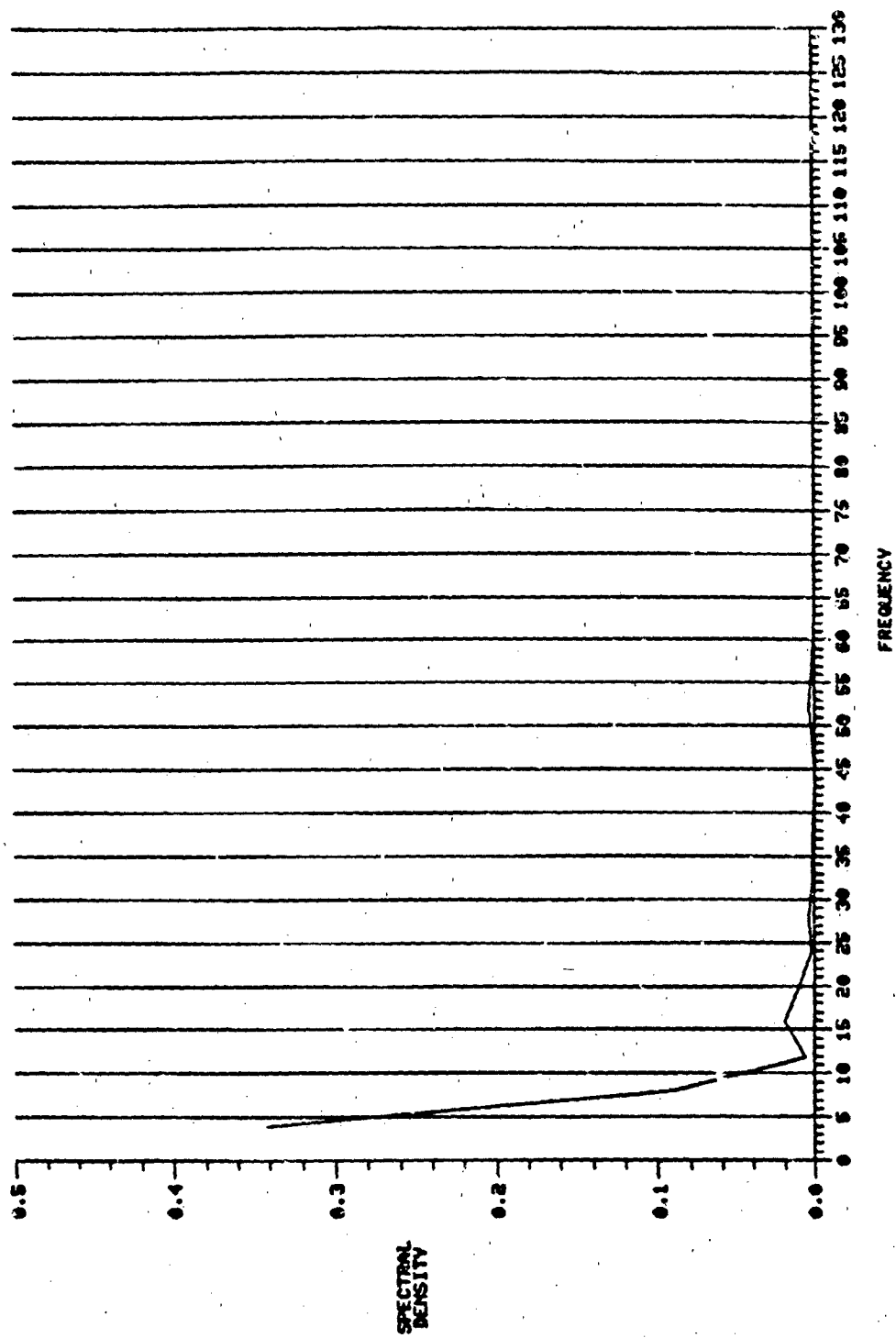


Figure 47. Spectral density function at fifth window of Figure 42.

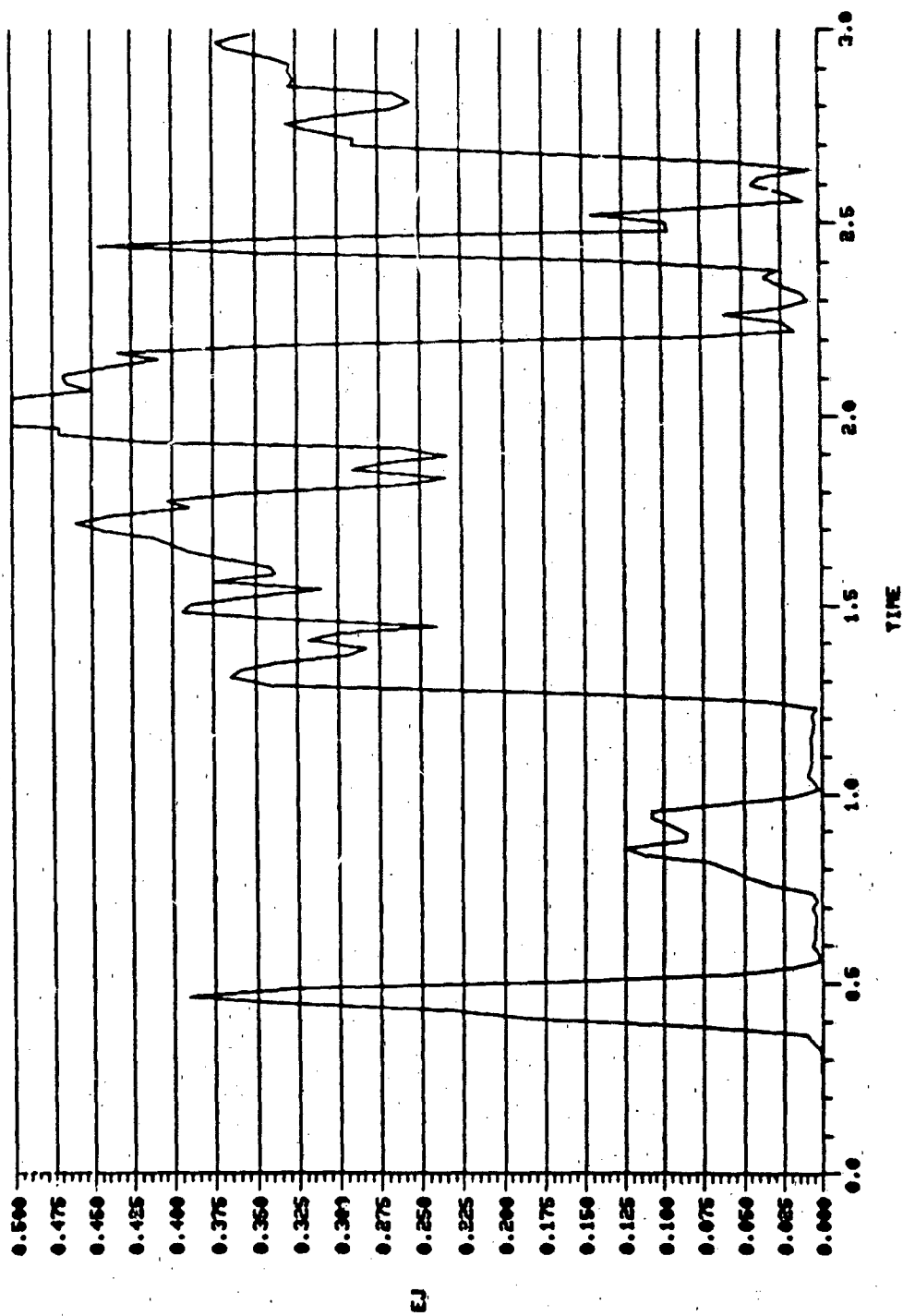


Figure 48. Error function versus time in data epoch for $\beta = 1.0$, neutral stimulus.

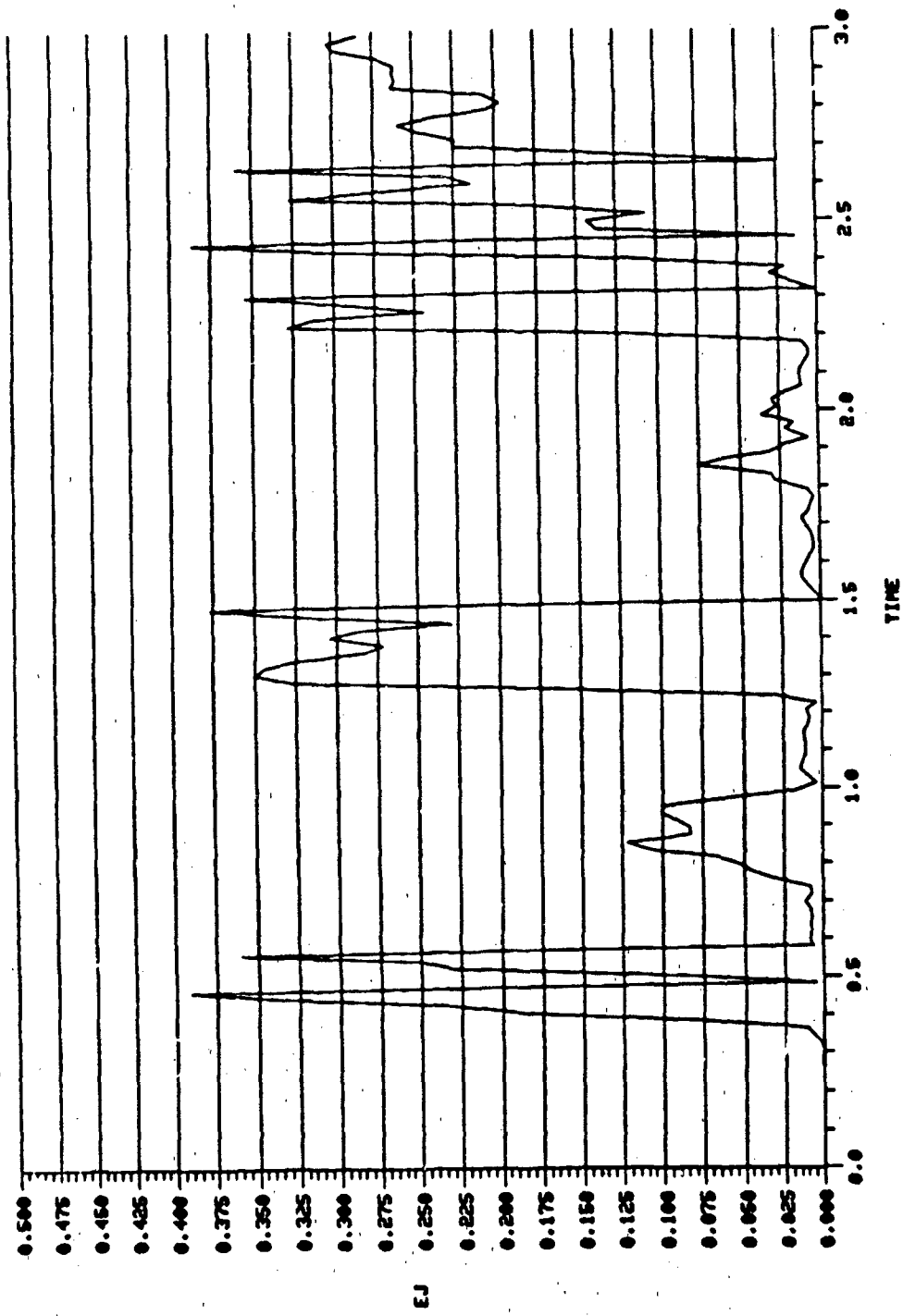


Figure 49. Error function versus time in data epoch for $\beta = 0.35$, neutral stimulus.

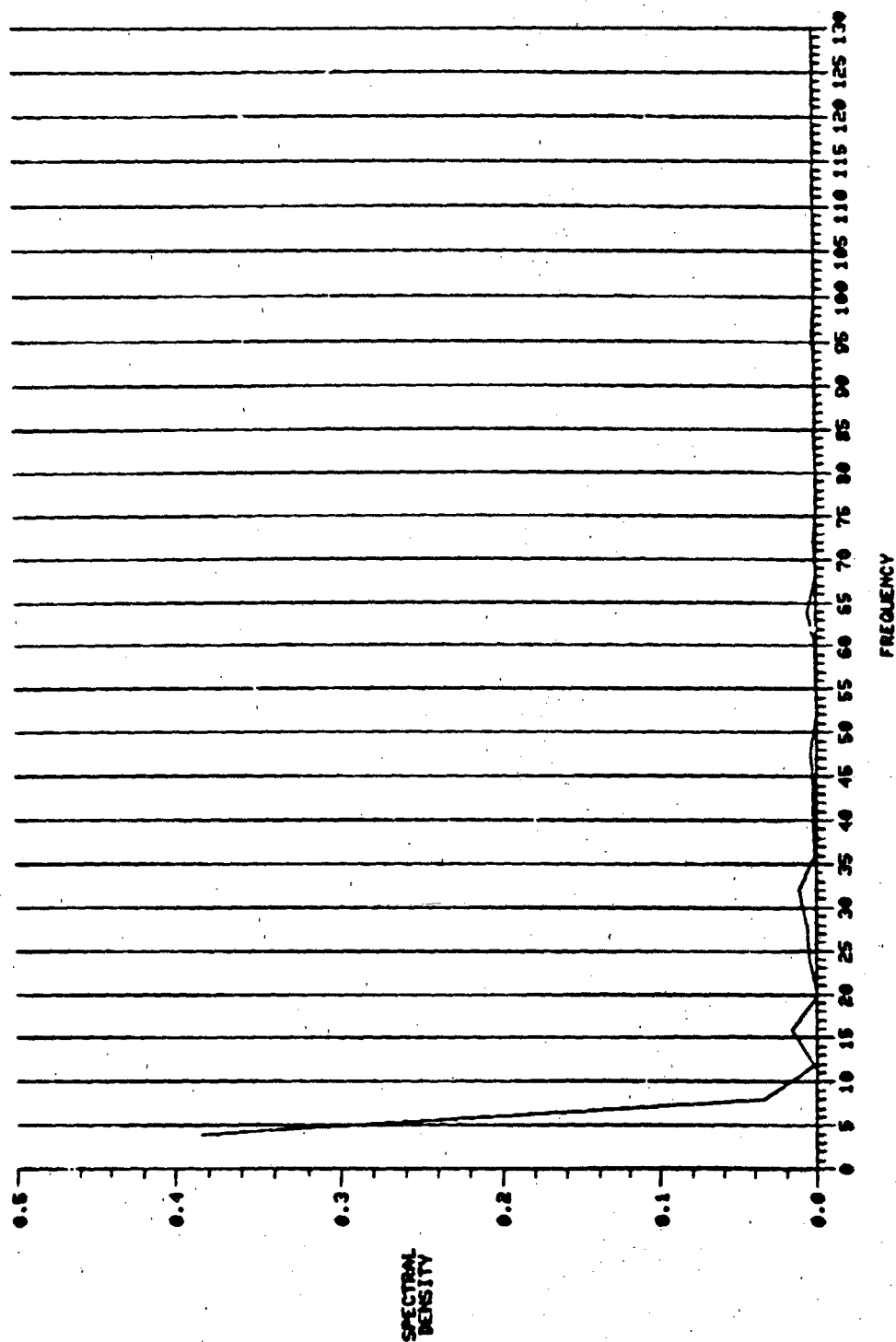


Figure 50. Spectral density function at first window of Figure 49.

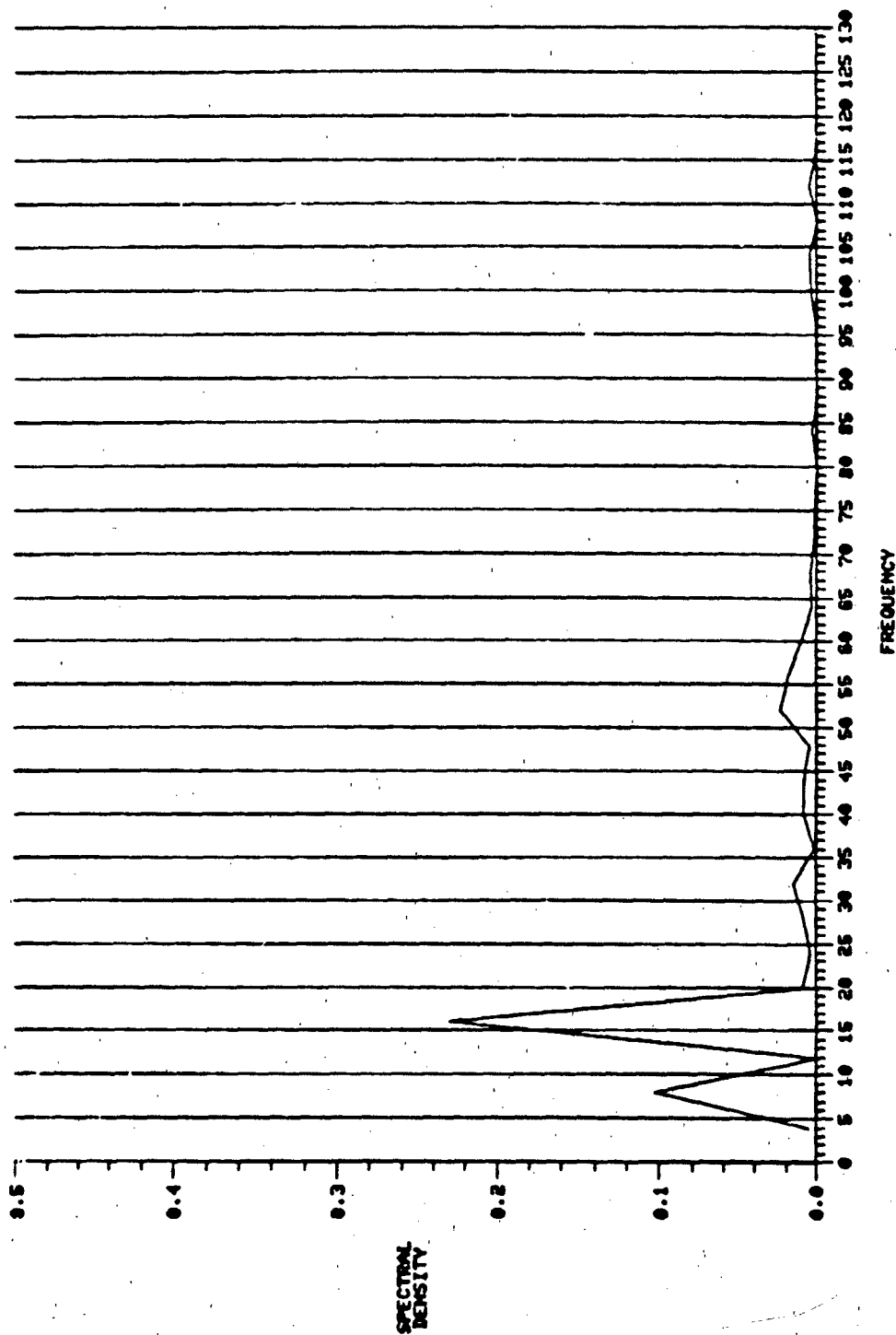


Figure 51. Spectral density function at second window of Figure 49.

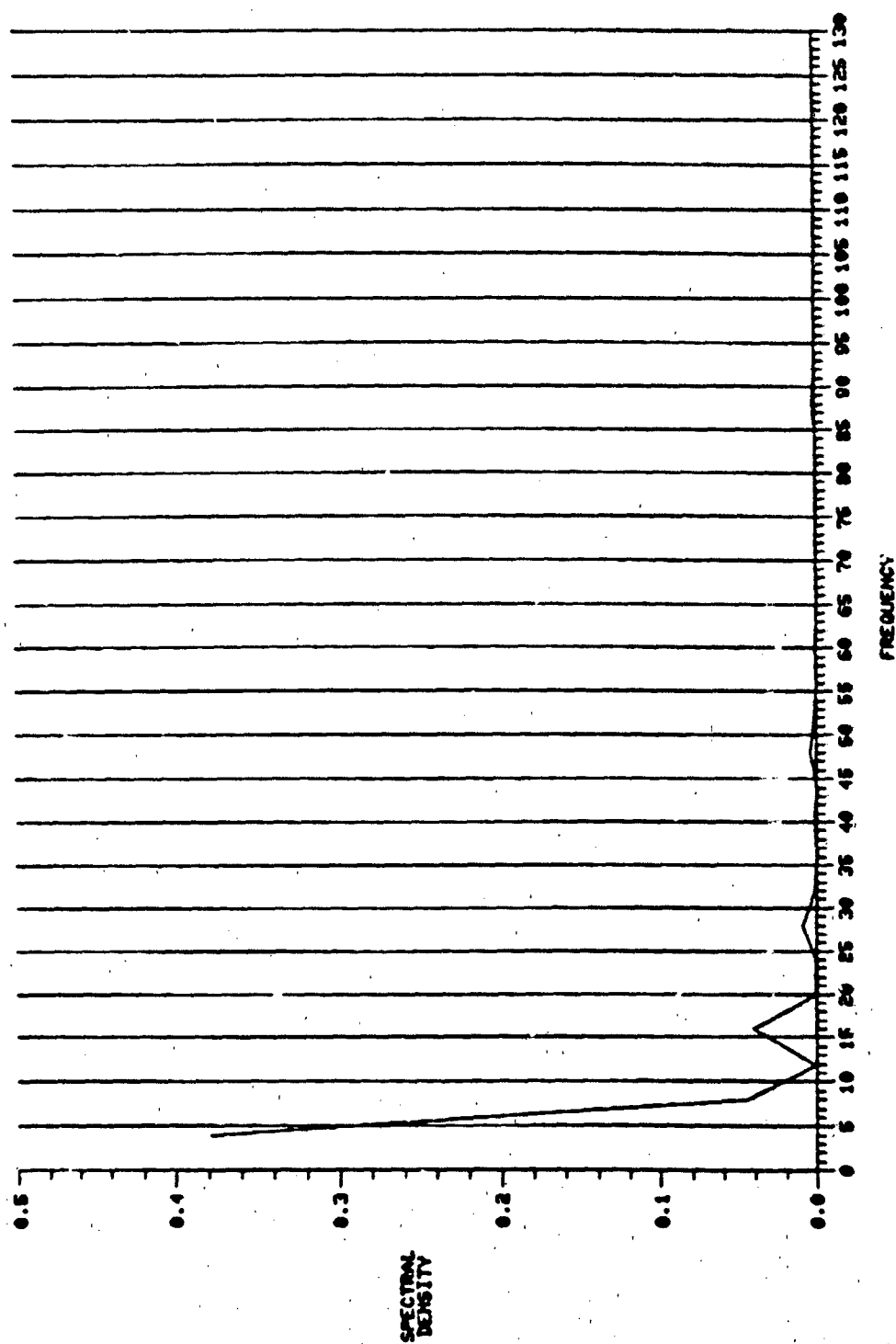


Figure 52. Spectral density function at third window of Figure 49.

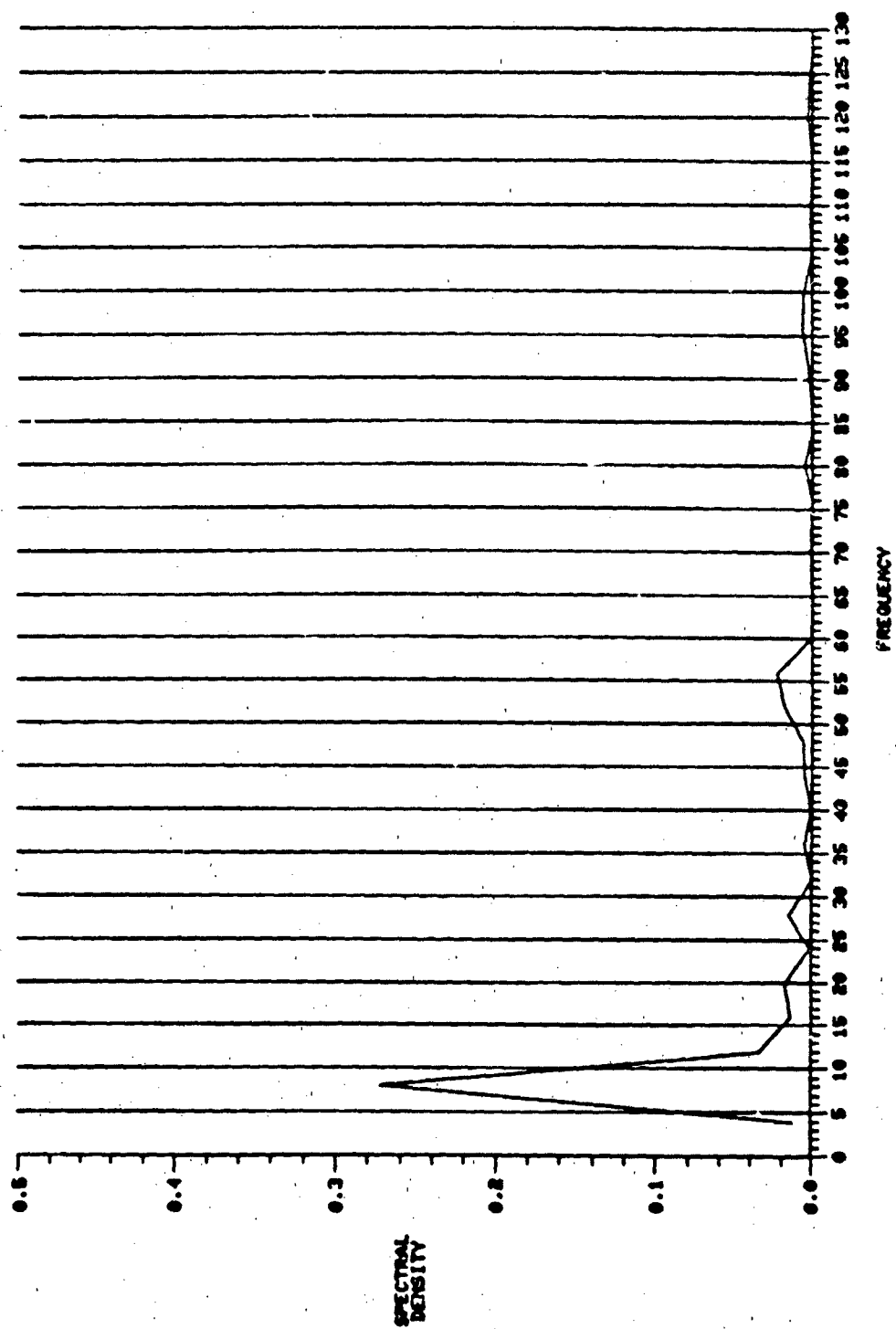


Figure 53. Spectral density function at fourth window of Figure 49.

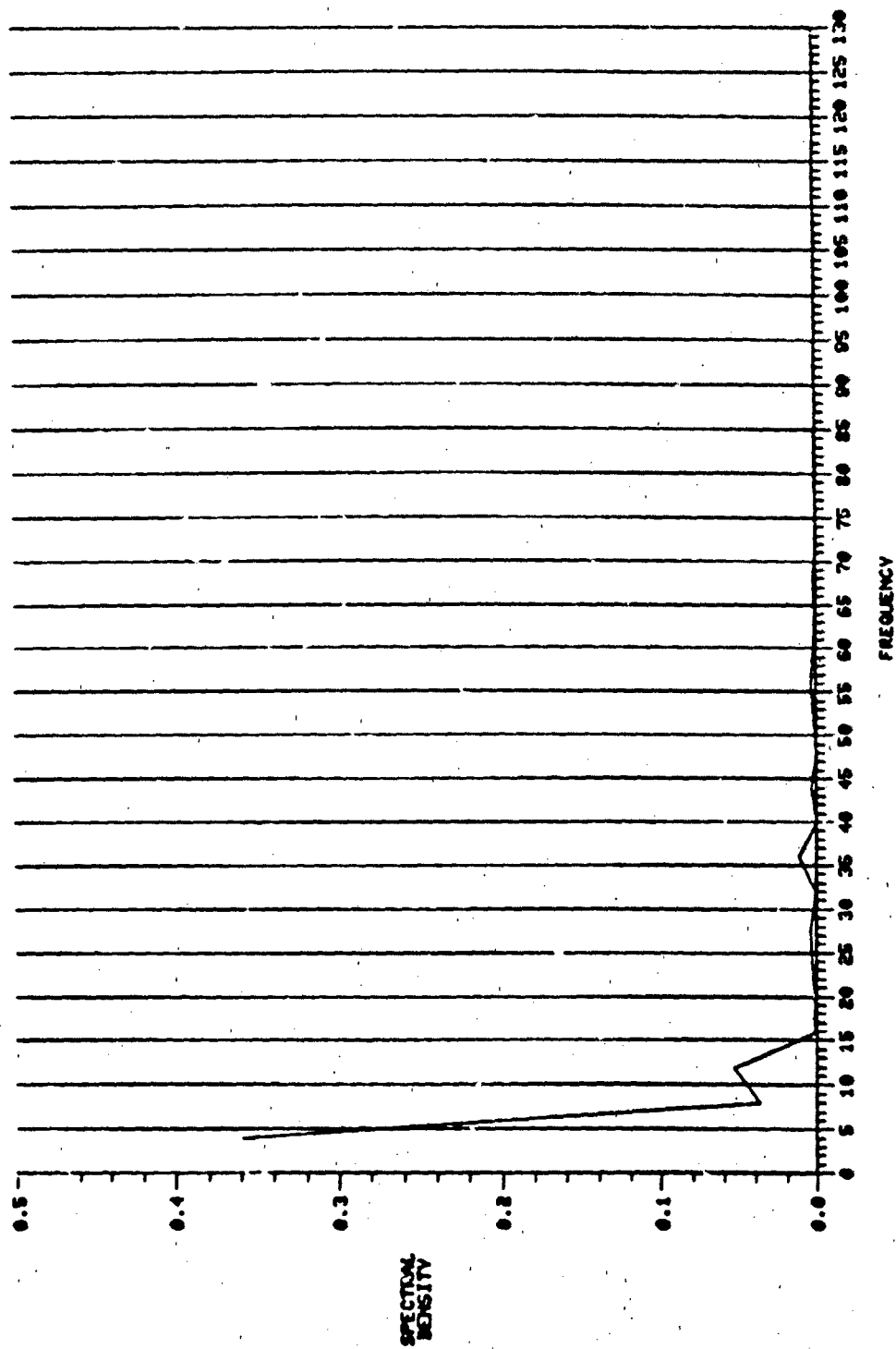


Figure 54. Spectral density function at fifth window of Figure 49.

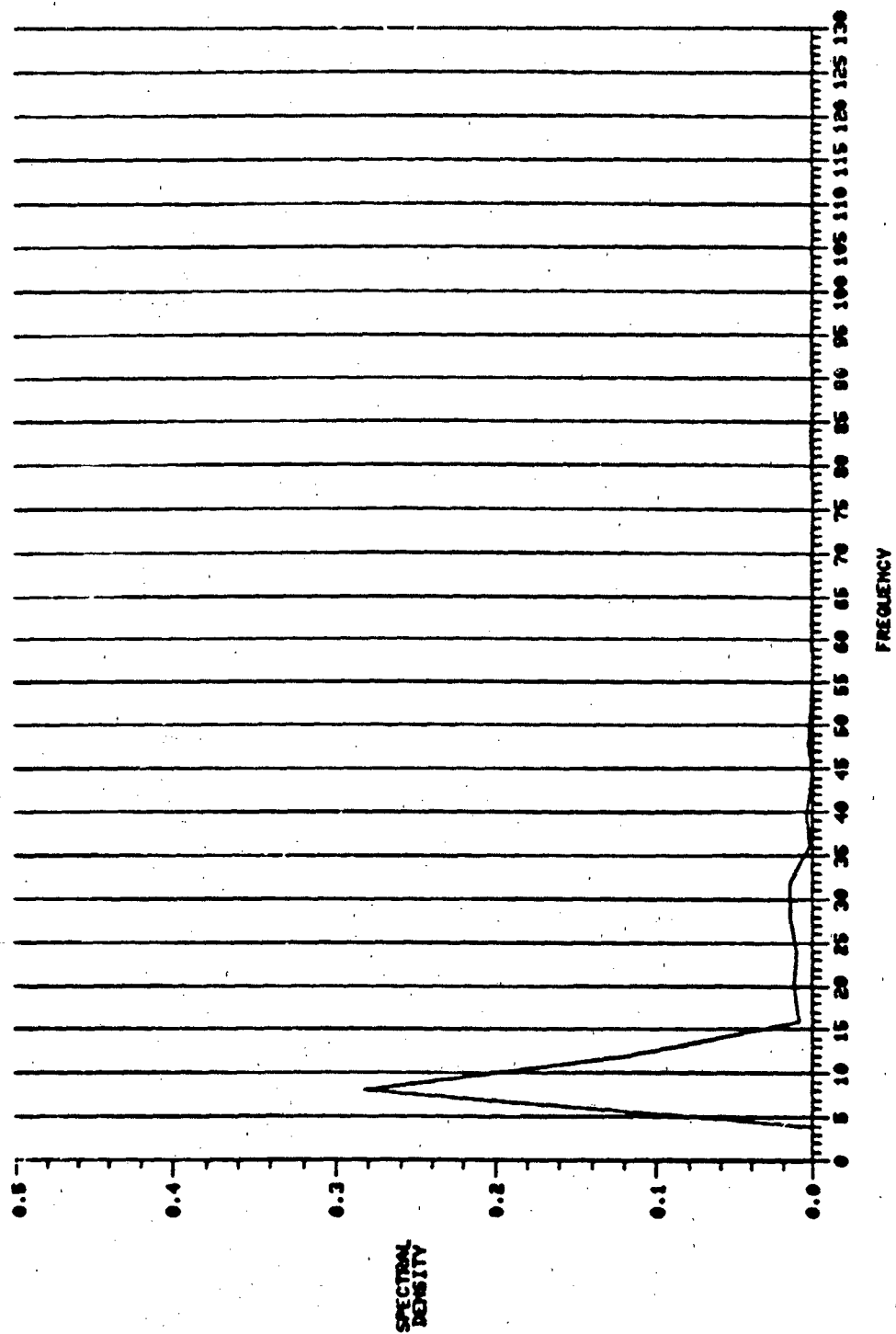


Figure 55. Spectral density function at sixth window of Figure 49.

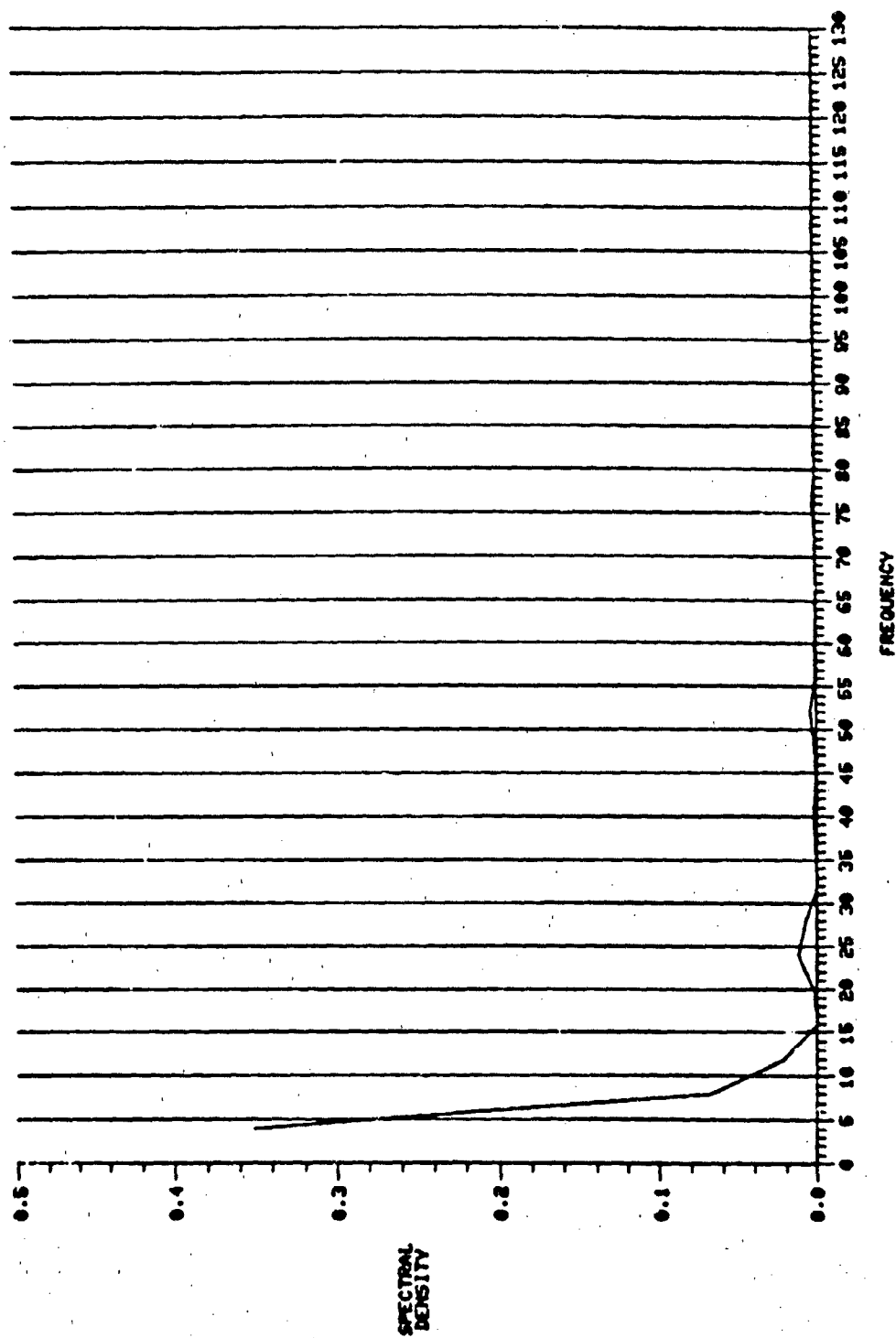


Figure 56. Spectral density function at seventh window of Figure 49.

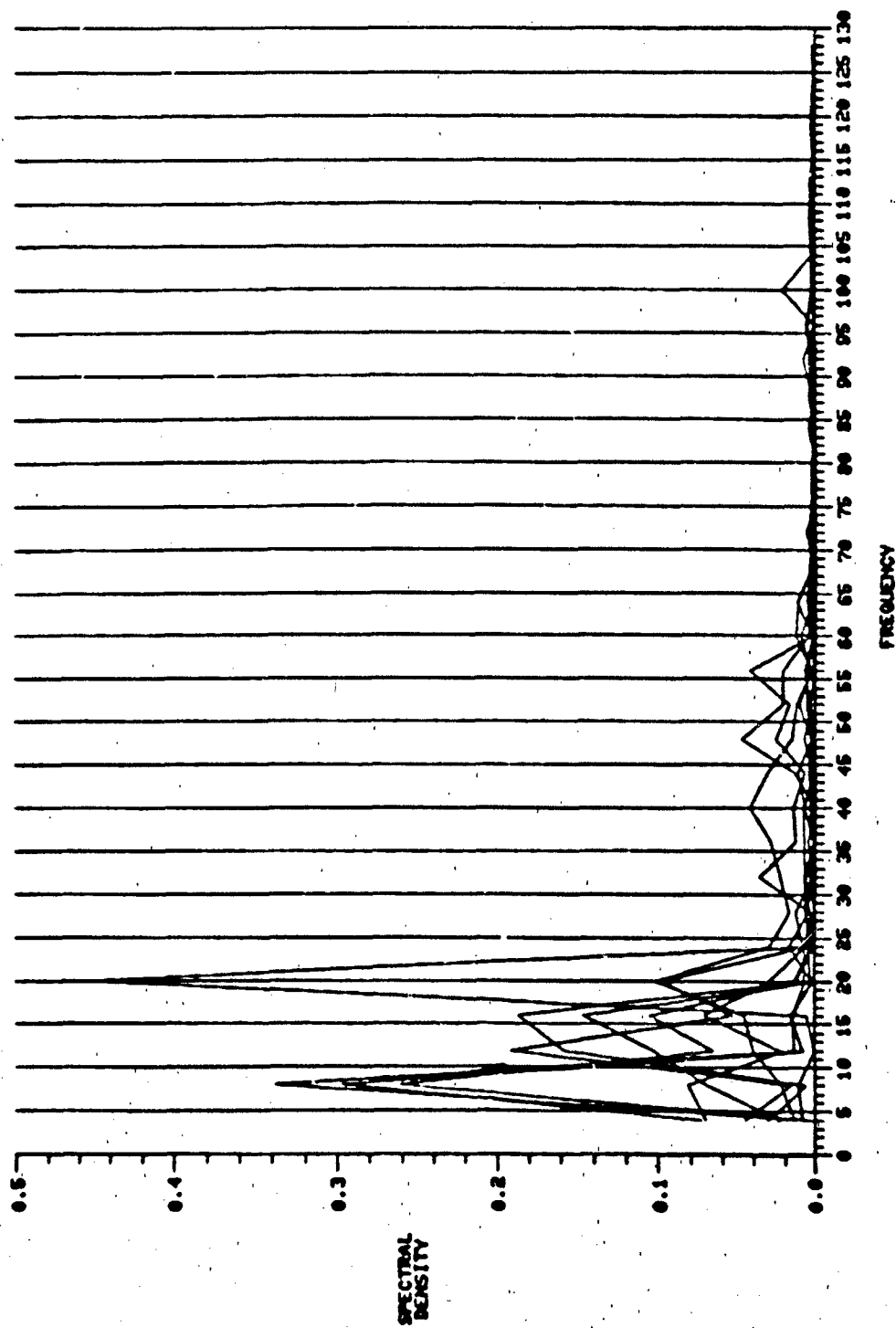


Figure 57. Composite spectral density for all rats, TNT stimulus.

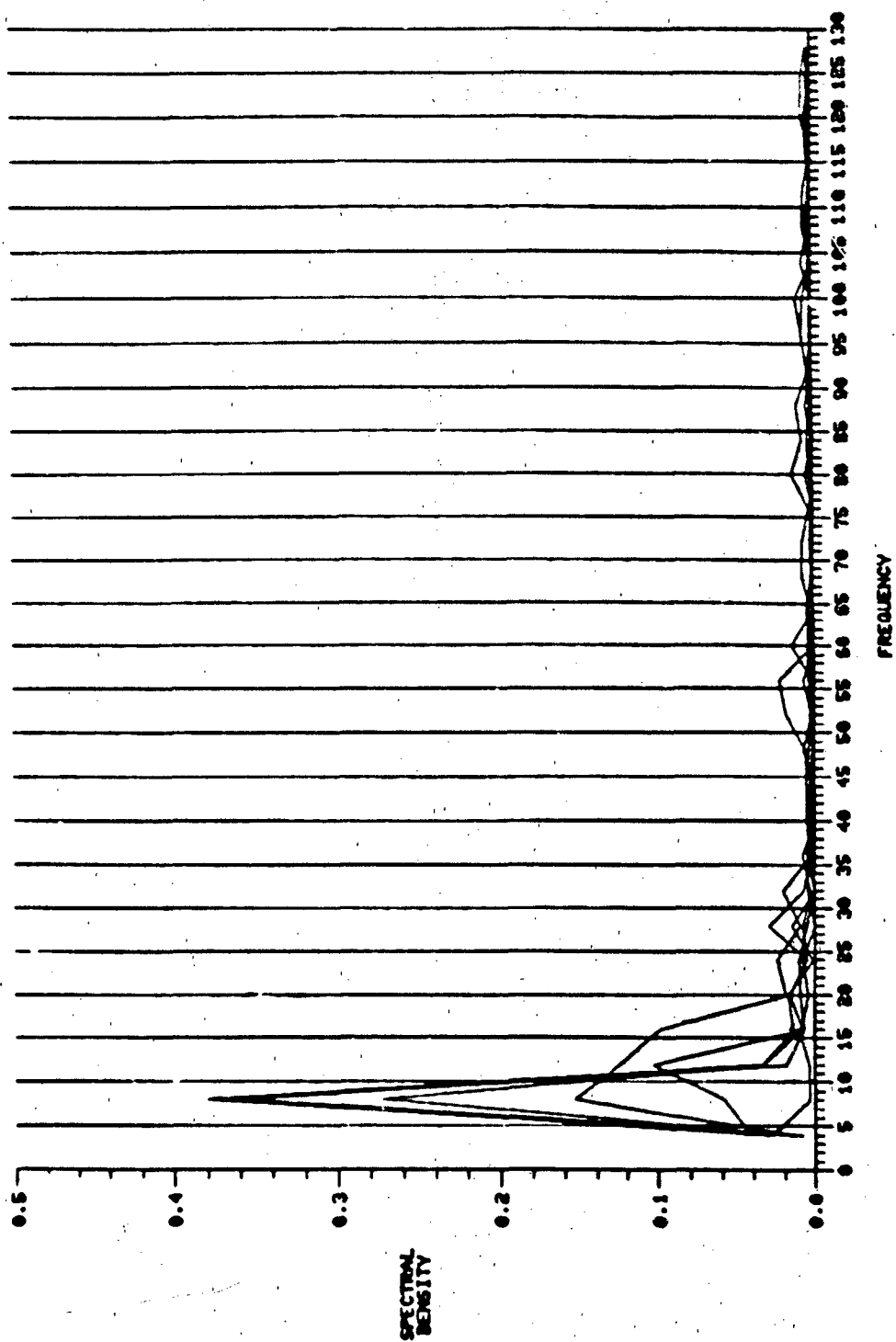


Figure 58. Composite spectral density for all rats, neutral stimulus.

Figure 44 is a plot of the spectral density function at 1.5 s where W_0 was first reset as the threshold was dropped to 0.175. The peak value of 16 Hz (which occurs in the Beta band in EEG conventional terminology) seen here is consistent with the mammalian state of intense alertness, as defined in numerous texts on the subject of electroencephalographic interpretation.⁹¹⁻⁹⁴

Figure 45 shows the spectral density function for the event seen at 1.7 s in Figure 42. Again, the animal appears to be at rest even though he has recently received a TNT odor bolus which obviously excited him. This behavior could represent the realization that EBS was not forthcoming, but this "explanation" is pure conjecture and merits no particular credence.

Figures 46 and 47 show frequency spectra at 2.5 s and 2.7 s. It should be noted that no further iteration of this pattern was seen in the data past 3.0 s in the few instances where this search could be conducted.

Figure 48 is the neutral-stimulus homologue of Figure 41. As before, W_0 is first located at 0.25 s with $\beta = 1.0$. The large values of F_1 in this plot suggested a β of 0.350 which resulted in the plot of Figure 49. Figures 50 through 56 show the nature of the spectral density at each new window location across the epoch, as suggested by the peaks seen in Figure 49. Since the number of variable factors which could disturb any individual experiment are legion, it is important to present the ensemble of data across all rats for the segmentation process, as was done with the Covariance Process. Figures 57 and 58 achieve this goal.

Here are displayed the composite plots of the individual spectral density functions for the subjects A, B, C, G, 97, 98, 100, and 103. Each figure depicts the spectral nature of the events occurring in the windows closest to 1.5 s (0.5 s post-trigger) across the ensemble.

⁹¹ Davidoff, L. M. and Dyke, C. G., "The Normal Encephalogram," Lea and Febiger, Philadelphia (1951).

⁹² Ehl, R., "Randomness and Synchrony in the Generation of the Electroencephalogram," In: Petsche, H. and Brazier, M. A. B. (eds) Synchronization of EEG Activity in Epilepsies, Springer-Verlag, New York (1972).

⁹³ Gibbs, F. A. and Gibbs, E. L., "Atlas of Electroencephalography," Vol. I, Addison - Wesley, Reading, Massachusetts (1958).

⁹⁴ Gibbs, F. A. and Gibbs, E. L., "Atlas of Electroencephalography," Vol. III, Addison - Wesley, Reading, Massachusetts (1964).

⁹⁵ Hill, D. and Parr, G. (eds), "Electroencephalography," Macmillan, New York (1963).

⁹⁶ Kitch, L. G., McGonias, A. J., and Osseken, J. A., "Clinical Electroencephalography," Appleton - Century - Crofts, New York (1972).

⁹⁷ Kim, K. A., "Fundamentals of Electroencephalography," Harper & Row, New York (1971).

⁹⁸ Shaw, J. C. and Oglesby, C., "The Measurement of Synchronization," In: Petsche, H. and Brazier, M. A. B. (eds) Synchronization of EEG Activity in Epilepsies, Springer-Verlag, New York (1972).

In Figure 57, the Segmentation Process elucidates two dominant peaks and one apparently spurious peak. The significant peak at 8 Hz may possibly represent those physiological processes associated with the so-called Alpha rhythms in mammals (8 to 13 Hz) or it may have a more obscure meaning here. The peak at 20 Hz occurred in one subject only and may be an artifact which would be insignificant in a larger data sample. The most significant peak in this figure is that existing between about 10 Hz and 17 Hz. Lesser peaks are seen to appear out to nearly 60 Hz with TNT stimuli data.

Figure 58 also manifests a dominant peak at about 7 Hz to 8 Hz, and it probably has the same significance as it does with TNT data plots. A secondary, broad peak centered at about 10 Hz is seen as well as small peaks out to 125 Hz.

The significance of the spectral density function patterns beyond 18 Hz cannot be explained in terms of current knowledge of physiological function versus EEG structure in mammals, but the gross differences which so clearly exist between the TNT and neutral ensembles beyond 18 Hz argue almost as strongly for the existence of an AESC as do the manifest differences which occur below 18 Hz.

Clearly, the AESC appears in the plots of Figure 57 primarily as the frequency/density peak seen between the 8-Hz cluster and the single 20-Hz peak. The composite structure is so different from that generated by the neutral stimulus data presented in Figure 58, that the broad peak in Figure 57 must be called unique, especially when it is coupled with the 18-Hz to 60-Hz peak structure and when the entire structure is then compared to the neutral ensemble!

This, then, is the final argument in support of the postulate of Thesis D. Two totally different processes—covariance and segmentation—present strong evidence of the presence of unique cortical spectral features occurring in time coincident with the application of TNT olfactory stimuli in the specially trained test population. Taken individually, each process presents a good argument for the occurrence of the AESC; taken collectively, there is little doubt that an identifiable feature does exist as a direct consequence of the TNT stimulus, and there is little vestigial doubt that this feature can be identified by existing microprocessor technology, especially if three or more experiments are summed before recognition is demanded in the detection system. One may assume, therefore, that Thesis D is proved to be valid.

g. Signals or Noise. When signal feature extraction is as difficult to achieve as that experienced in this research program, there may be some doubt as to the exact nature of the plots which are presented to show signal features. The question, "Are these merely plots of the covariance of random noise, or do they actually represent the covariance of special signal features which relate to the conditioned response to a specific olfactory stimulus," begs to be answered.

Figures 59 and 60 answer the question graphically, and in so doing, attest to the authenticity of Figures 25 through 39. Figure 59 is the plot of 25 1-s intervals of random noise sampled at 512 data points. This figure is the random-noise equivalent of Figure 40, which displays the raw analog EEG signals of 25 experiments using Rat C as the test subject. Figure 25 is the plot of the $\Delta 11$ covariance means of the data of Figure 40. Figure 60 displays the plot of Figure 25 as the bottom trace, while the plot of the $\Delta 11$ covariance means of the noise samples seen in Figure 59 is displayed as the top trace.

Clearly there are great differences between these covariance plots in both amplitude and structural detail. The difference in average amplitude implies that the degree of covariance of the noise samples is much less than that of the EEG signals, while the gross structural differences relate to differences in the degree of stationarity between the two data sample sets.

The difference of about three to one between the auditory peak of the EEG in the lower trace and the highest peak in the noise covariance plot in the upper trace is evidence of the great degree of covariance of the aperiodic EEG signals which are time related (throughout all the EEG data) only to the solenoid valve sound. Other peaks in the EEG covariance mean values exceed the highest noise covariance peak by a considerable amount, and these peaks must represent some (undefined) physiological quasi-stationary event arising from the aforementioned "housekeeping" cortical and skeletal neural activity.

Another salient structural difference in the curves of Figure 60 is the obvious difference in signal bandwidths. The EEG plot indicates the absence of high-frequency covariance, and it has been stated earlier that the maximum frequency of the cortical spectrum was found to be generally lower than 64 Hz, even though the data system bandwidth was 200 Hz, and probably in most epochs the maximum frequency of significant power was about 40 Hz.

The noise covariance plot gives evidence—in its fine structural detail—of much different frequency distribution. This is not unexpected, since, at a sampling rate of 512/s, the maximum unambiguous frequency present can be 256 Hz with totally random distribution of amplitude—and thus we may expect the noise covariance to reflect this spectral configuration.

One must conclude from these and other observations that the covariance plots for EEG signals assuredly do not have the characteristics of the covariance observed with random noise.

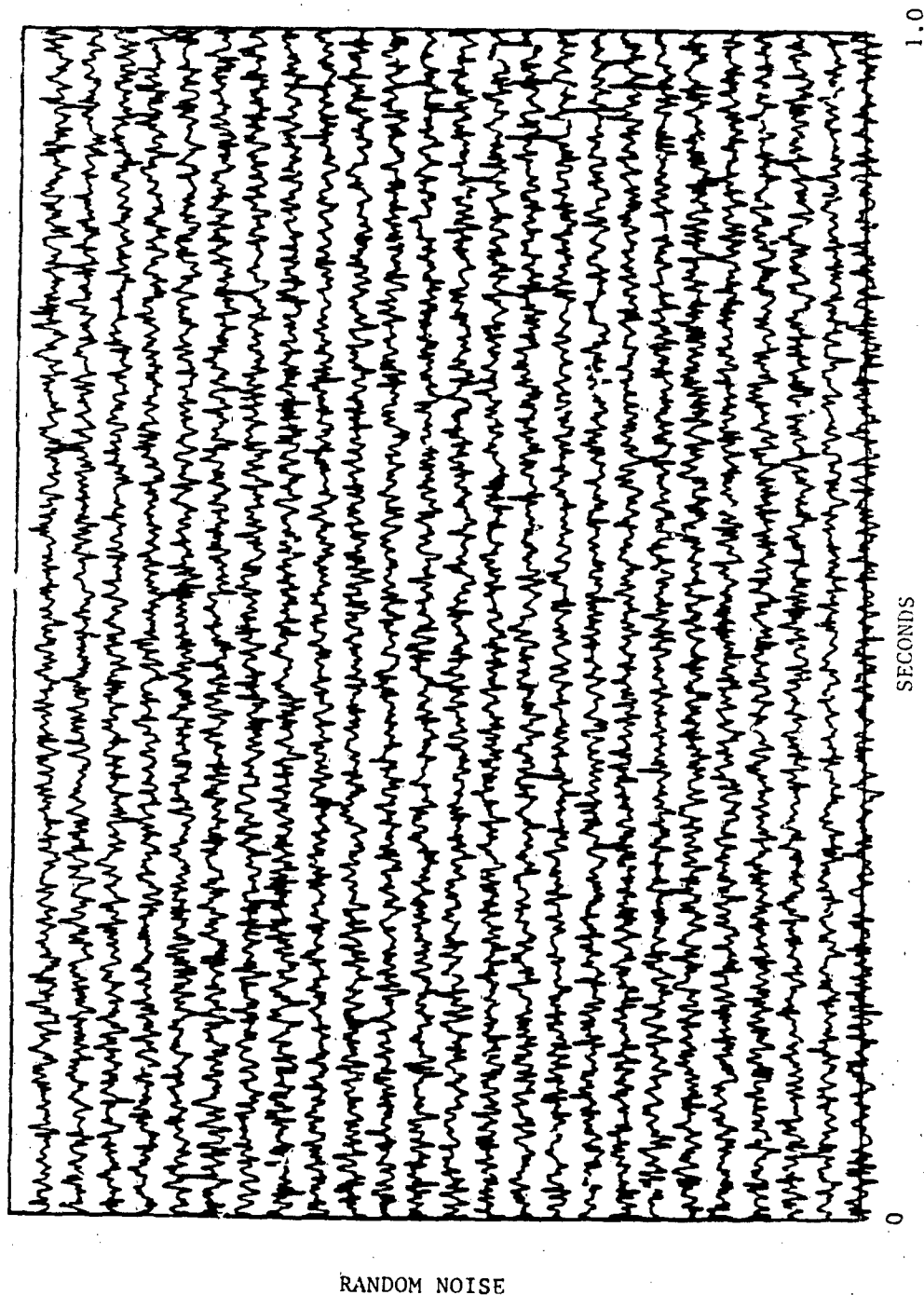


Figure 59. Twenty-five 1-s random noise epochs.

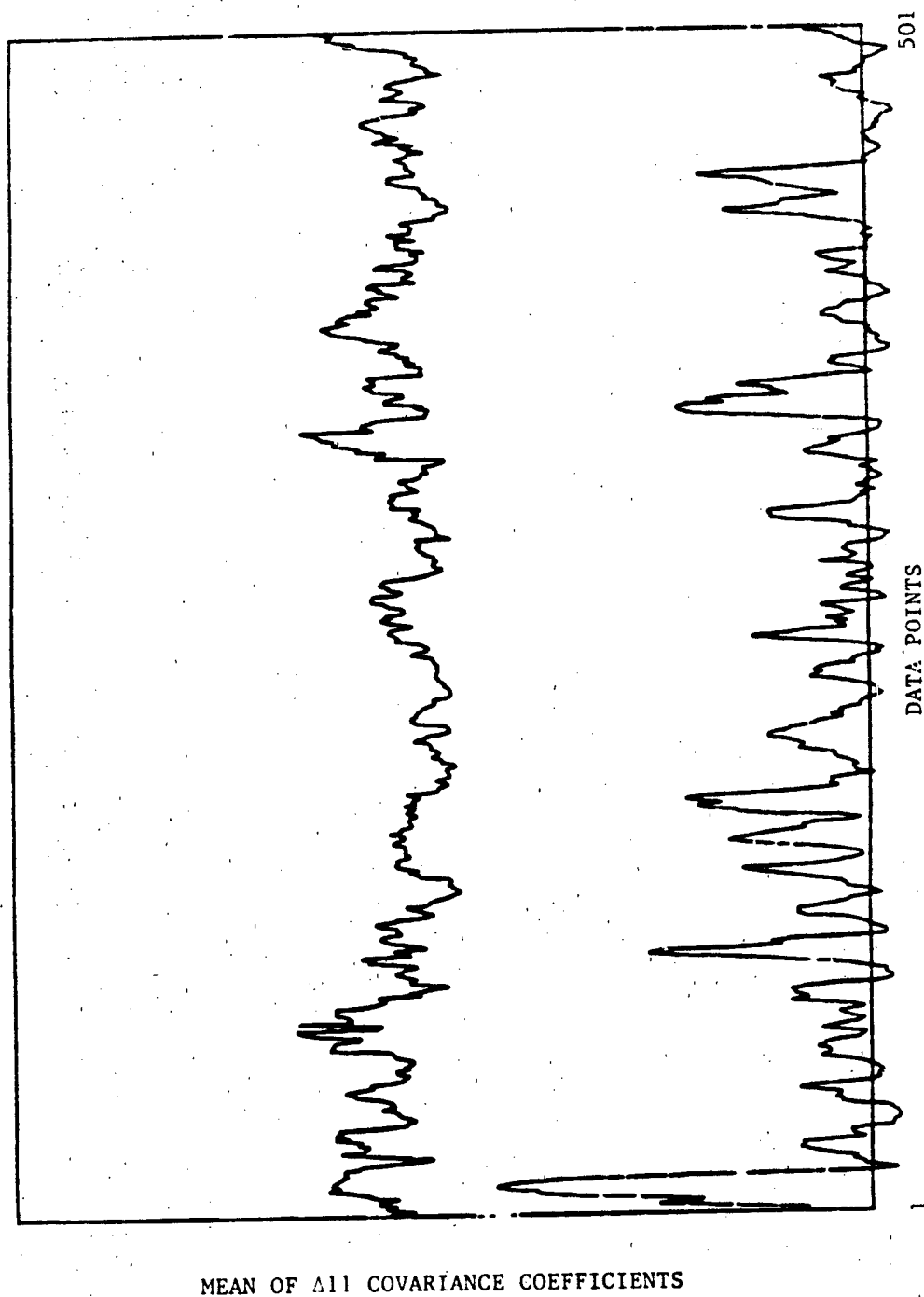


Figure 60. Comparison of $\Delta 11$ covariance of 25 random noise samples and 25 EEG epochs for Rat C, TNT stimulus.

Similar observations as to the non-random nature of the EEG are also in order for the Segmentation Process. Figure 61 displays the error function, E_j , versus time of two signal samples. The upper trace displays the E_j of random noise over the 3-s interval with $\beta = 1.0$. As noted in the discussion of the Segmentation Process, the predicted and measured value of β for random noise is 0.0155 and this relationship is evident here where the E_j plot displays virtually no peaks of visual significance.

The bottom trace of Figure 61 is the plot of E_j for an EEG experiment from the data for Rat C. Here, there is ample evidence of the non-randomness of the rat EEG, over short periods of time. These plots when augmented by the plots of Figure 60 are convincing proof that the results of the experiment are based upon physiologically significant events and are not the result of mathematical operations upon random occurrences.

Even though the plots of covariance and segmentation appear to argue incontrovertibly in favor of the proof of Thesis D, it was decided to apply a single statistical test to one set of data as the coup de grace. Since the segmentation process was the final metric used, the data were conveniently accessible in the MERADCOM computer, and thus these data were subjected to the test.

The particular test applied was the standard Wilcoxon signed rank test, which is fully described in many texts. This technique is a relatively simple nonparametric test which will determine whether or not a set of paired values manifests a significant mean difference. Here, the word "significant" may be used to indicate that sufficient (or insufficient) evidence exists in the tested data to accept (or reject) the hypothesis. For the TNT data from the segmentation process, the "tail" probabilities indicate significant values ranging from 0.005 to 0.05, which clearly demonstrate the extreme unlikelihood that the data would be as they are if only random events occurred in the 1-s interval following the trigger.

Much of the data taken during this long series of experiments has yet to be analyzed. When—and if—all remaining data are examined by the covariance and segmentation process, it seems highly likely that the statistical evidence will be weighted even further toward solid proof of Thesis D, simply as a result of the greatly increased number of samples.

h. Experimental Controls. The nature and extent of the experimental controls appears to be most appropriately treated as the final argument in support of the stated results after all experimental data have been fully discussed, and for this reason the rationale appears under Section III, "RESULTS," in this report.

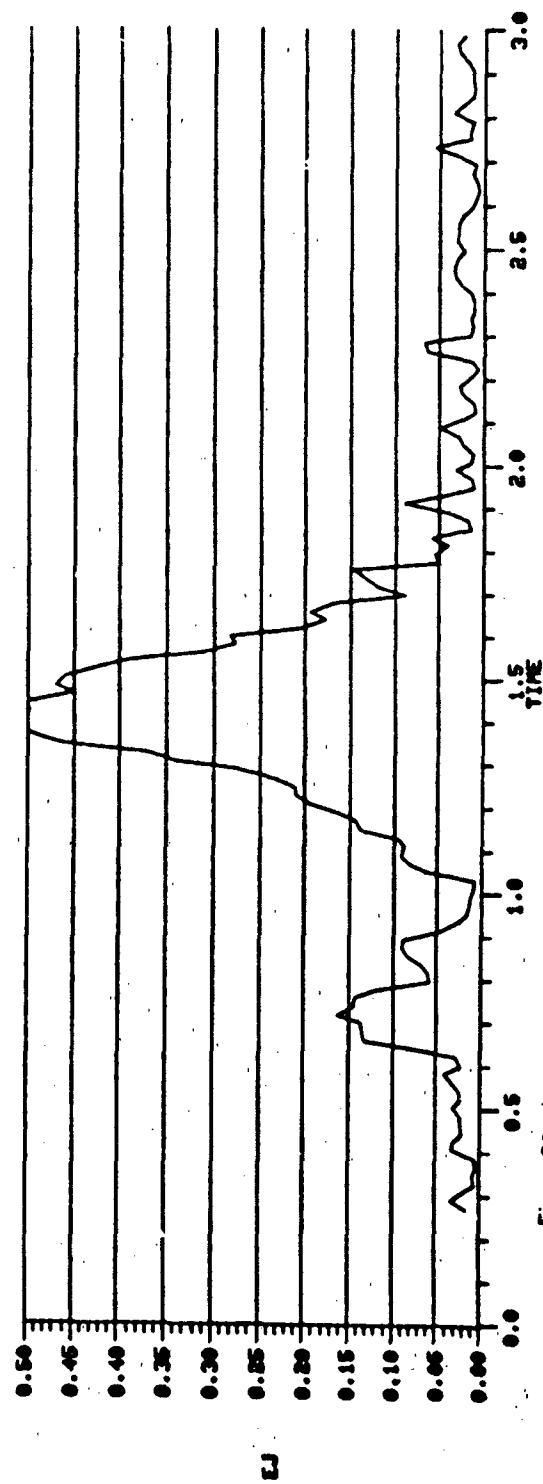
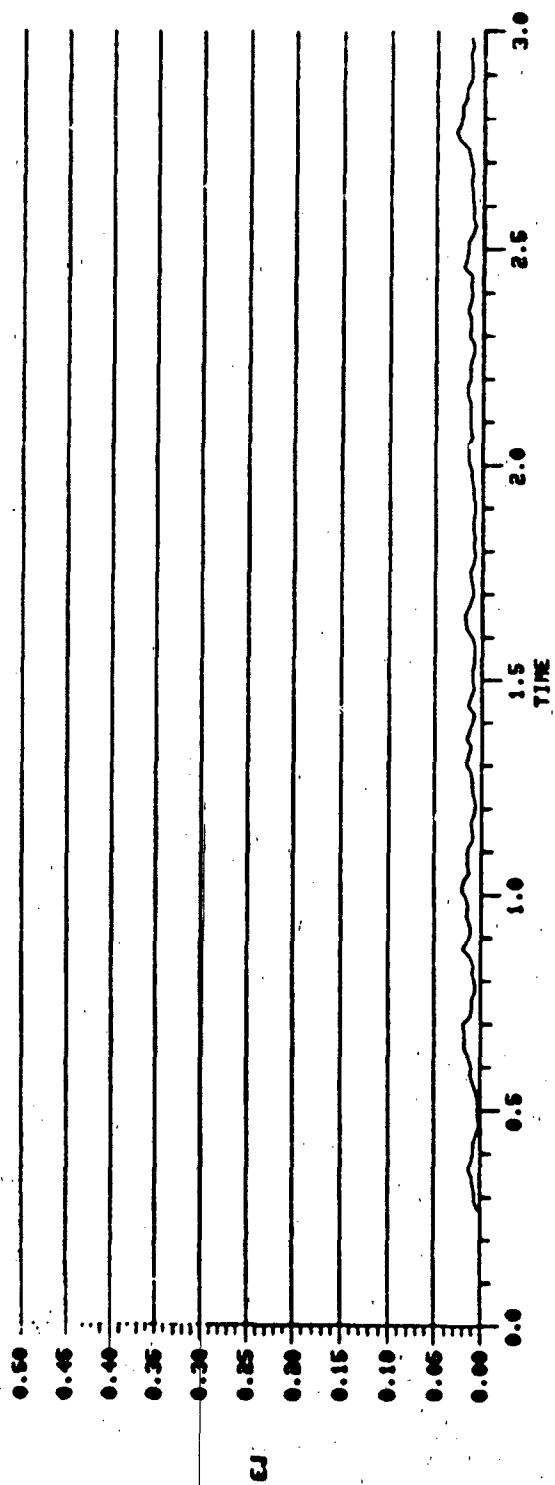


Figure 81. Error function versus time of random noise sample and EEG epochs for Rat C, TNT stimulus.

While it would perhaps have been possible to concurrently maintain two populations of experimental subjects for the purpose of comparing the surgically and behaviorally modified rats at each step in the research program to a totally and continually naive control group, the additional factors of time, expense, and data complexity were judged to be, in all, of negative value to the program. For this reason, the use of a continually naive control group per se was excluded from consideration early in the program.

However, the test group population was, itself, suitable to serve as a totally naive control group by use of the EEG recordings derived prior to test protocol implementation from each surgically modified subject. It was always necessary, as explained in Section II, "METHODS AND MATERIALS," to ascertain that the EBS and EEG electrodes were properly located and functional prior to use of any rat as a test subject.⁹⁹ Thus, the term "naive" applied throughout the test population refers to naivety with regard to the pairing relationship of EBS to TNT vapor presence and not to ignorance of the emotional phenomena associated with simple EBS.

To accomplish this control function, each test participant was placed in the test fixture and subjected to random application of TNT and neutral olfactory stimuli while the EEG was continuously recorded. As might be expected, little of consequence was derived from these naive-subject data. There was, a priori, no reason to expect a unique feature in the character of the EEG signals derived during periods of unfamiliar olfactory stimuli, and, in fact, no repeatable, discernable feature was observed in the EEG signals a posteriori across all naive rats when they were exposed to TNT vapor. Also, there was no behavioral evidence which might indicate that TNT was a significant olfactory stimulus at any time prior to TNT/EBS pairings.

Figures 62 and 63 show the $\Delta 11$ covariance means for one totally naive subject—Rat 100. The highest of the peaks near the left edge are believed to be the result of long-latency processing of the solenoid valve relay acoustic signal. Generally, the same (approximate) peak location was observed across all naive rats, but the individual variation was large, both with respect to the peak amplitude—and hence to the degree of covariance—and with respect to the latency. It was interesting to observe the change in the nature and position of the auditory event as the subject was brought from the naive state to an awareness of the likelihood of EBS when TNT vapor was present. As with other observed physiological and behavioral events, there will be no discussion of the possible reasons for the change in this event, since it is inappropriate here. Suffice to say, there was generally a shift in the temporal position of the auditory event from a naive-subject location at perhaps 60 ms to 70 ms post-trigger to the 40-ms "final location" in fully trained subjects. These plots, for the $T\phi$ and $N\phi$ interval covariance means of 10 experiments, were chosen at random from a collection of similar figures.

⁹⁹ The reader will recall that EBS must be applied in the full-recovery, post-surgical state to confirm the correct placement of the electrode in the MFB. EEG data are also observed in this test phase to determine the patency of the contact between the surface of the dura mater and the EEG electrode, and the patency of the cap connections.

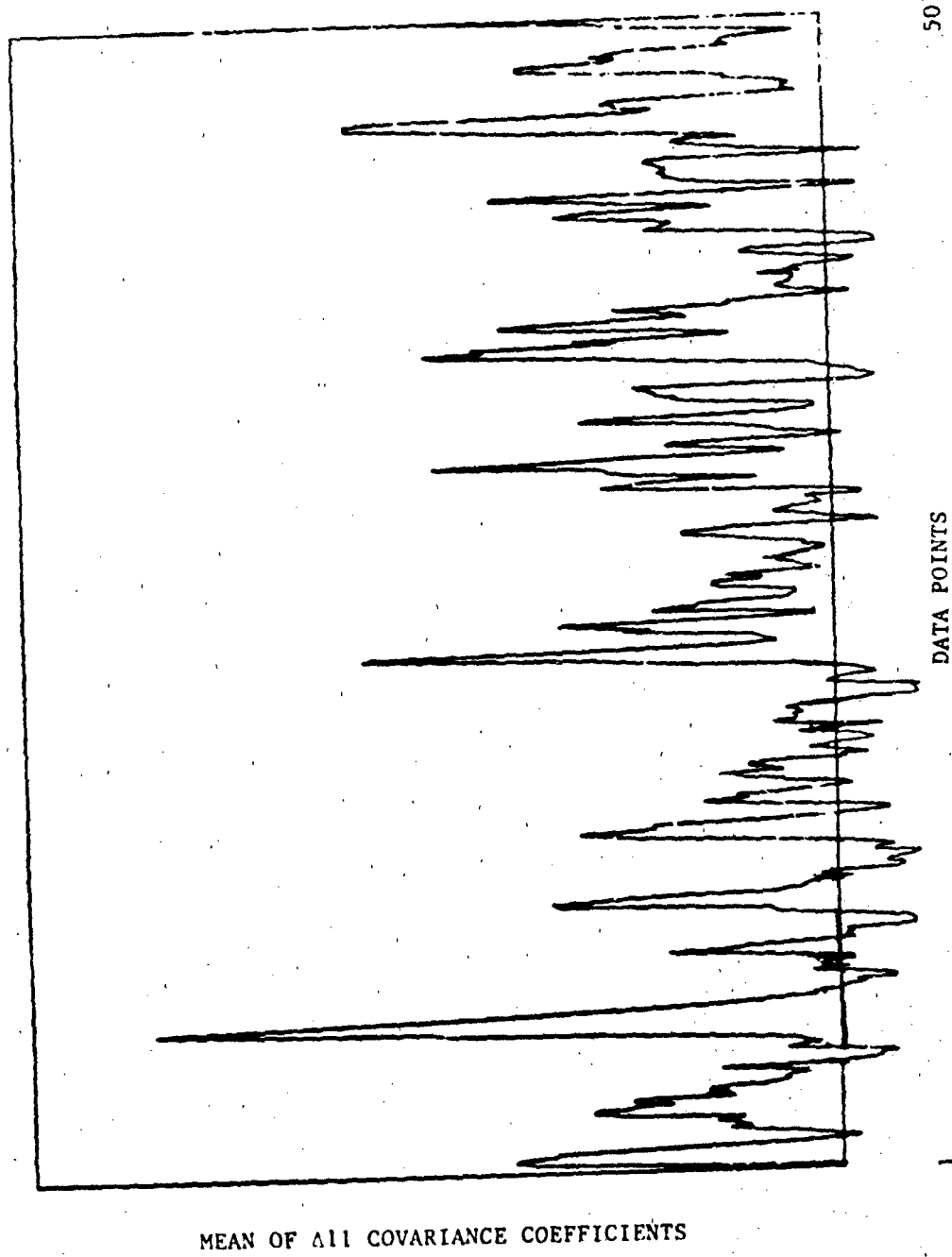


Figure 82. Mean of $\Delta 11$ covariance coefficients for naive subject. No interval, Rat 100. Ten experiments.

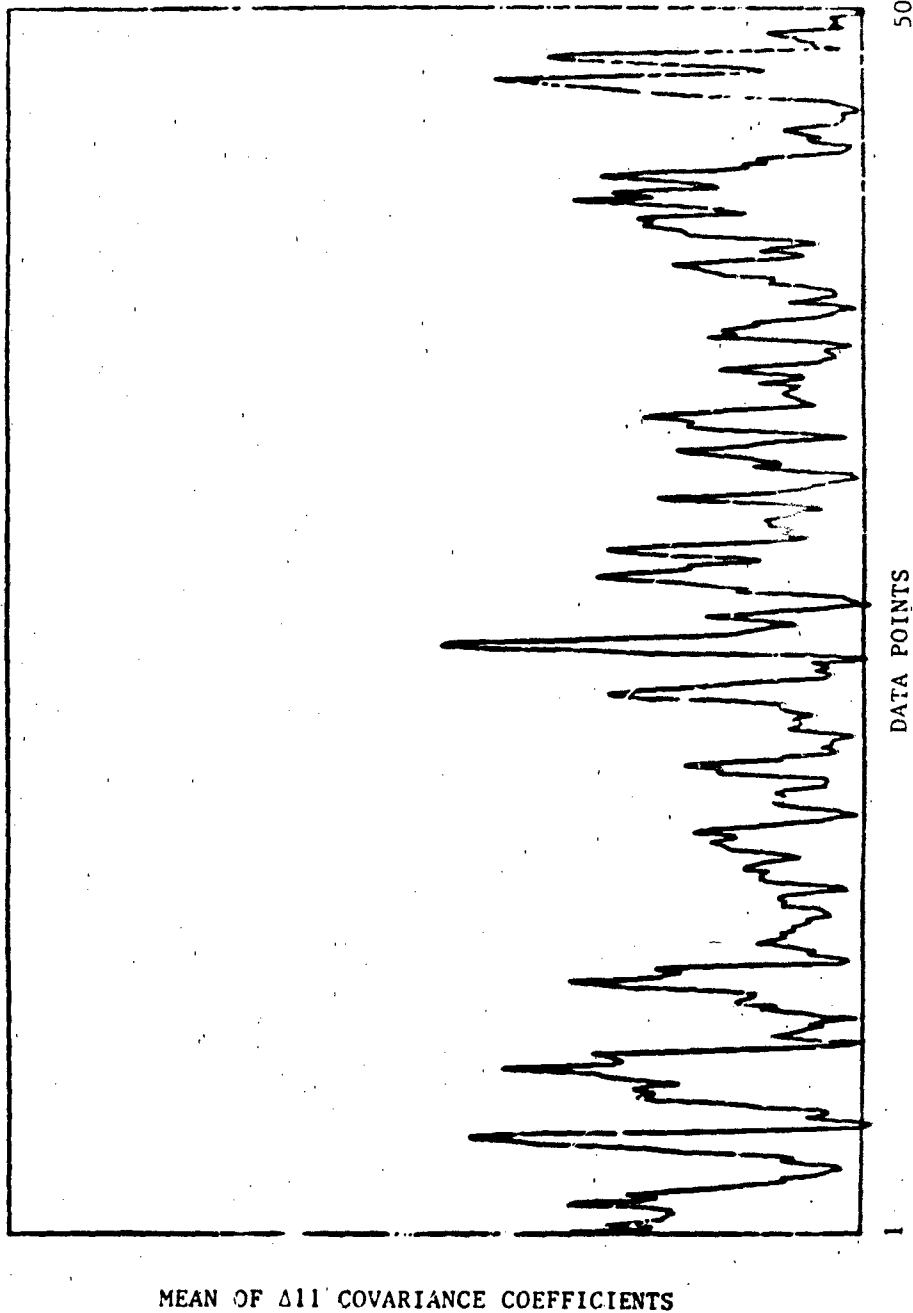


Figure 63 Mean of $\Delta 11$ covariance coefficients for naive subject. $T\phi$ interval, Rat 100. Ten experiments.

Except for the evidence of a rudimentary auditory event, there is nothing remarkable to be seen in these plots, nor was there anything of consequence to be seen in the plots for $T - 1$, $T + 1$, $N - 1$, and $N + 1$ for this naive rat. Examination of many similar plots offer convincing arguments to the effect that there is nothing inherent in the experimental protocol which, of itself, causes the characteristic changes observed in the $T\phi$ EEG of the trained test subjects. Therefore, it appears certain that those features just described, which appear uniquely with the presence of TNT, are indeed due to the emotional response of a trained subject to the potential arrival of an EBS reward. The representative figures (62 and 63) certainly show no evidence of any profound or "characteristic" covarying event to odorant presence such as that seen in Figure 32.

Additionally, it must be recognized that the fully trained subject population served as controls across the various time intervals examined in data processing. In those series of events where TNT was the olfactory stimulus, it was always evident that the covariance process background EEG interval, $T - 1$, was, in essence, a control period for comparison with the $T\phi$ interval, as was also the case for the $T + 1$ interval. The same was true for $N\phi$ comparisons with the $N - 1$ and $N + 1$ intervals. Further, the neutral events in all intervals ($N - 1$, $N\phi$ and $N + 1$) served as control data in the search for the unique event in the $T\phi$ interval.

While the events portrayed in Figures 62 and 63 are based upon the Covariance Process, the remarks are, in general, true for all metrics, and thus they apply in concept to the Segmentation Process. For the sake of completeness, however, Figures 64 and 65 are included to show the output of the Segmentation Process to the naive data of Rat 100. As with the plots of covariance, there is no discernible evidence that the naive rat is affected by the presence of TNT as is the trained rat.

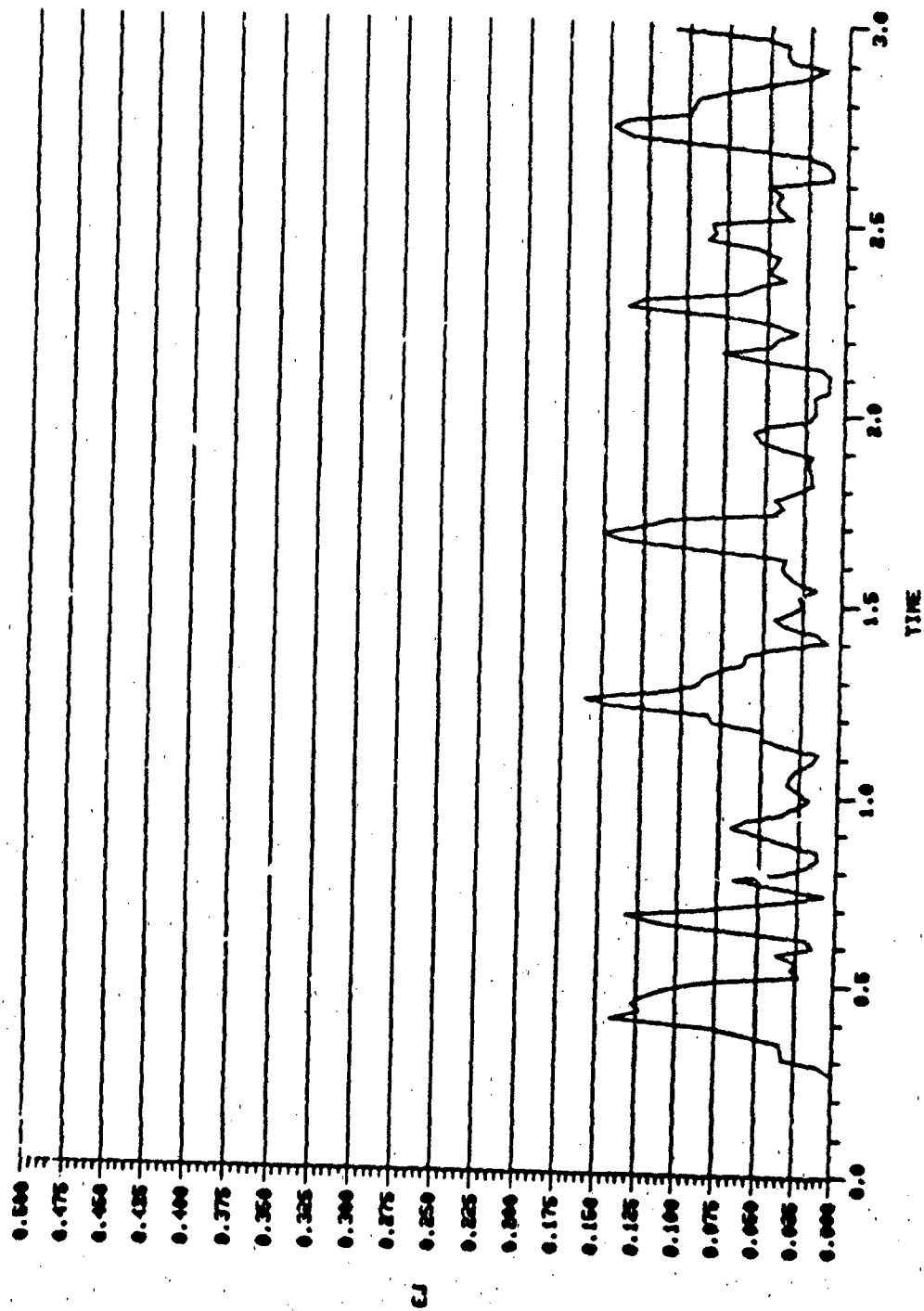


Figure 64. Error function versus time in naive rat 100 data for $\beta = 1.0$, TNT stimulus.

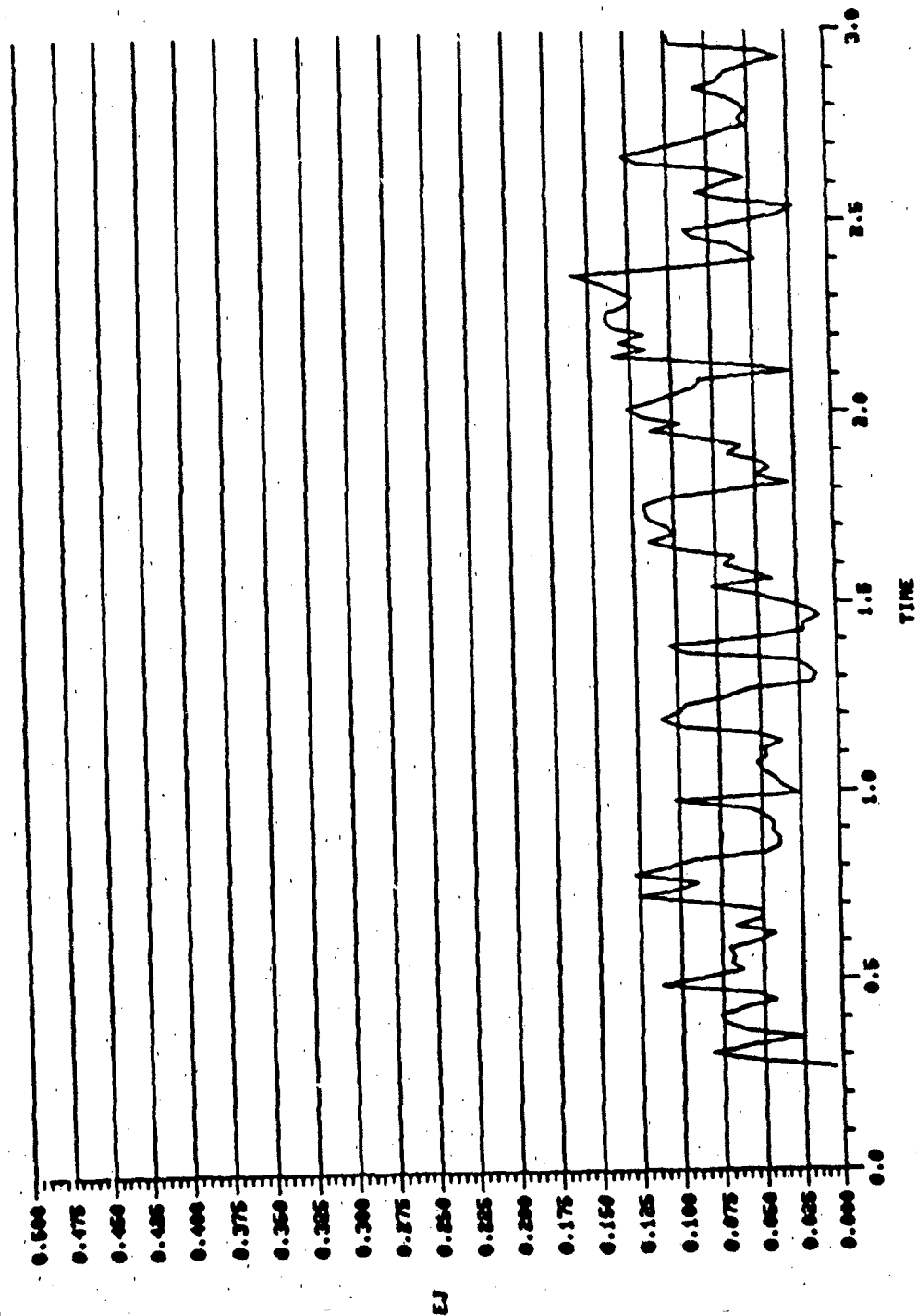


Figure 65. Error function versus time in naive rat 100 data for $\beta = 1.0$, neutral stimulus.

IV. SUMMARY AND CONCLUSIONS

29. Summary. As noted in Section I, one of the most promising research domains is the largely unexplored area of biosensors wherein both *in vivo* and *in vitro* applications of the sensory functions of living creatures may be used as sensory elements in new detection systems. Biosensors using a living creature as the sensor—the *in vivo* approach—offer the best chance of near-future application in light of current knowledge. In this approach, the practical research goal is to achieve the optimum animal/machine interface, which is defined as that operational configuration which results in optimum sensitivity and specificity while maintaining a reasonably high true response to false response ratio (10 to 1 or better).

The research program discussed herein was the first significant step in this direction. It is clearly evident from the data presented that albino male rats of the Sprague-Dawley strain can reliably detect some characteristic olfactory signal from the effluents of military-grade 2,4,6-trinitrotoluene when the effluent concentration is very small. This program may perhaps best be defined as the successful opening gambit in a series of research efforts directed toward the use of composite rat/microprocessor systems for the detection and annunciation of the presence of extremely low concentrations of a variety of target substances. As stated in the text, no effort was made to quantify the minimum detectable concentration across the test subject population.

There was no effort in this program to identify odorant targets other than TNT which the test subjects might detect because—as is the case with sensitivity—the effort is vast and complex and far beyond the intent of the instant research.

It must be stressed that the field of biosensor detection research is in an embryonic state where every effort appears to be largely a pioneer effort with little prior knowledge to serve as guide posts. Hopefully, the success of this research program will spur competent researchers in both the public and private sectors to delve deeply—and quickly—into the methods for optimal use of animals as detection system sensors.

Briefly, the instant research program sought to prove that rats could detect TNT vapor via the olfactory sensory modality and that they could be trained both to signal the fact of detection by operant means—a treadle press—and by evidencing a distinctive change in their cortical EEG at the moment of detection. Prior to achieving this goal, it was necessary to demonstrate unequivocally that rats could detect and operantly annunciate the presence of TNT vapor. Proof of the existence of a TNT-dependent EEG signal feature was complex, and over half of the research effort and funding was expended in this area.

Several mathematical approaches were applied to the raw analog and digital EEG data, with each approach becoming more sophisticated. The final elucidation of the elusive Anticipatory Evoked Spectral Change was achieved by two processes which are quite different in nature.

The first successful process employed the covariance relationship between similar events, whereas the second successful process compared the error function existing between significant events in power spectra across a data epoch. Selection of the best microprocessor program to perform these functions in the suggested rat/microprocessor configuration was not a research goal, and hence, no recommendation is made as to which technique is better for the ultimate purpose of explosives detection. Such decisions must await the outcome of future research—and neither technique may be chosen for the first field-test system, if the continuing search for more efficient metrics is effective.

30. Value of the Research Effort. The net value of the research to the sponsoring agency is, at this point, perhaps indeterminate. While it has been demonstrated that small laboratory animals can, in fact, indicate the presence of a target substance with no conscious action on their part, the gap between this achievement and a practical detection system for man-portable service is wide and formidable. However, there are now no known technical barriers which cannot be crossed by ample research. Given adequate funding—always the most uncertain aspect of research—it is rational to anticipate field testing of a portable rat/microprocessor device within a 5-yr period. Deployment of field grade devices might require another 5 yr past initial testing, in view of Army development cycle scheduling.

31. Future Research. It is important to include herein a general outline of the immediate steps which should be followed to continue the effort. The areas for immediate investigation are:

a. Signal analysis by the covariance process should be expanded to include "dithering" of the existing data from individual experiments to determine the limits of variation of physiological and experimental latency of response from experiment to experiment and from animal to animal.

b. The remaining unexamined data from this pioneer program should then be analyzed, using both covariance and segmentation, to the point of diminishing return. These residual data include the monopolar signals derived from the parietal and occipital electrodes, plus the bipolar data derived from the various lead combinations (parietal-cingulate, parietal-occipital and cingulate-occipital). While it is somewhat doubtful that these data contain more significant information than that obtained from those cingulate electrode data segments used in the instant research, it would be inconceivable not to examine these data prior to undertaking further animal experimentation.

c. While the covariance process and the segmentation process were successfully used to define the presence of an olfactory-induced AESC, it is logical to assume that other signal processing schemes may exist which could be used either in an ancillary function with one or both of these processes, or individually, to improve the effectivity of signal feature extraction. At present, a suitable source for such advanced methodology cannot be specifically identified but new computer algorithms appear more or less continuously in the vast and expanding area of signal processing, and possibly a continuing search will disclose a more appropriate algorithm for future EEG signal analysis.

d. In order to achieve effective detector performance in a real-world environment, the AESC must be elucidated from the data of no more than three or four successive 1-s EEG samples from any individual subject. To accomplish this goal, several major research steps must be made.

(1) The locus of the AESC must be defined more precisely than at present. This goal can probably best be achieved by a precise and orderly mapping of the brain structures of the Sprague-Dawley strain of laboratory rats used in the instant research.¹⁰⁰ Mapping should be extended to all areas above the mid-brain, beginning in the general structures known as the limbic system and progressing to the highest cortical structures. Isolation of a relatively small cell cluster (say < 1 mm) as the AESC locus would do much to raise the effective signal-to-noise ratio, and thus the process of AESC detection could possibly be resolved to a single EEG/olfactory stimulus epoch which, in turn, would result in a high reliability, real-time detection capability.

(2) Once the locus is generally known, all subjects can be tested to performance standards by automatic test equipment, as is standard practice with mass produced electronic circuit elements.

(3) Overall, a fully functional sensory element such as the rat/microprocessor combination could be "produced" in the main by automatic training and testing procedures based upon the results of the instant research.

¹⁰⁰ There is no valid reason for examining and mapping the brain in other strains, since the test subjects used throughout this program have evidenced no adverse characteristics.

BIBLIOGRAPHY

Note: The following bibliography was compiled both as background reference material for the research program described in the text and as a basic reference set for future experiments in biosensor systems. Since each citation has been examined and found to be pertinent to this research field, it would be an act of redundancy to compile an abridged list of references which would delineate only those citations from the total bibliography which are directly noted in the text. Thus, the text designation for direct references are the citation numbers found in this bibliography.

In the interest of completeness, however, it was determined that some mention should be made of the citations noted as specific references in the text. To achieve this goal, an asterisk was added prior to the citation number for the quoted references.

To simplify the task of examining the reference materials, the citations are grouped by the general subject matter addressed. Since many of the listed works address more than a single concept, a citation may appear in more than one grouping. As an example, citation 12 "A Comparative Study of the Olfactory & Trigeminal Reflexes Elicited by Various Vapors in Different Mannals," by W. F. Allen, appears in groups Ia, Ib, IIa, and IIb.

Those citations appearing in the "Miscellaneous" grouping are found only under that heading.

The groupings are ordered as follows:

I. Olfaction

- a. General olfactory physiology.
- b. Measurements.

II. Neurophysiology

- a. General physiology of the nervous systems.
- b. Stimulus — chemical.
- c. Stimulus — electrical.

III. Conditioning and Behavior

IV. Electroencephalography

V. Sensory Physiology

VI. Miscellaneous

Citations listed by Subject Matter.

Ia. General Olfactory Physiology.

4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 29, 32, 38, 42, 48, 58, 59, 69, 72, 80, 89, 92, 100, 111, 114, 121, 122, 123, 124, 130, 133, 135, 136, 139, 141, 151, 155, 156, 157, 159, 177, 182, 189, 203, 210, 211, 212, 213, 214, 215, 216, 217, 219, 241, 242, 251, 252, 253, 255, 256, 277, 278, 283, 289, 295, 296, 297, 300, 303, 315, 318, 319, 329, 330, 333, 339, 344, 359, 375.

Ib. Olfactory Measurements.

6, 7, 10, 12, 13, 15, 19, 21, 41, 73, 102, 130, 136, 151, 155, 156, 195, 209, 214, 215, 256, 277, 289, 317, 329, 333, 344, 345, 351, 361, 368.

Ila. General Physiology of the Nervous System.

2, 9, 11, 12, 13, 14, 15, 16, 21, 22, 24, 31, 35, 36, 37, 40, 44, 49, 51, 57, 64, 65, 66, 69, 74, 81, 87, 88, 89, 96, 97, 98, 103, 104, 108, 109, 111, 115, 116, 122, 123, 124, 128, 131, 132, 133, 134, 138, 142, 143, 146, 151, 154, 160, 161, 164, 168, 171, 172, 173, 174, 176, 178, 179, 183, 184, 193, 194, 198, 199, 202, 208, 210, 211, 218, 219, 223, 224, 225, 226, 227, 228, 229, 230, 234, 239, 241, 242, 244, 246, 247, 260, 262, 265, 266, 267, 272, 274, 279, 280, 281, 282, 284, 286, 292, 293, 302, 307, 308, 320, 324, 330, 331, 336, 340, 347, 349, 350, 353, 356, 357, 360, 365, 372.

Ilb. Stimulus-Chemical.

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IIc. Stimulus-Electrical.

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3, 4, 5, 6, 7, 8, 20, 29, 33, 38, 42, 59, 77, 121, 138, 139, 151, 182, 189, 192, 193, 194, 207, 251, 252, 318, 354, 369.

VI. Miscellaneous.

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